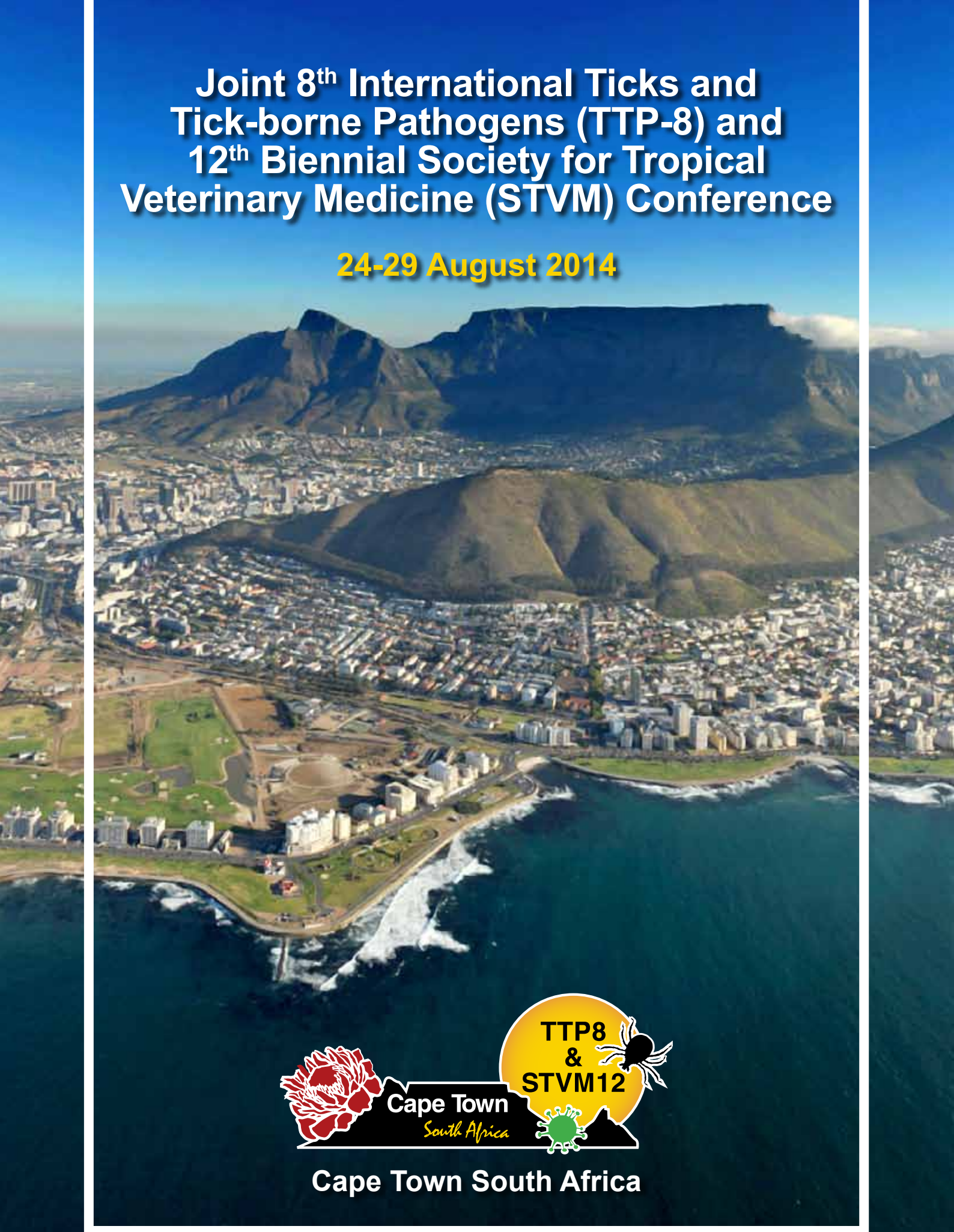
Joint 8th International Ticks and Tick-borne Pathogens (TTP-8) and 12th Biennial Society for Tropical Veterinary Medicine (STVM) Conference

24-29 August 2014



Cape Town South Africa

Poster Abstracts

POSTER SESSION I: MONDAY 25TH AUGUST (17H00-19H00)

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0157	**Emoke Pall:** Serological evidence for *Anaplasma phagocytophilum* presence in feral horses from Danube delta, Romania	
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0160	**Gabriela Lopes Vivas Vitari:** Molecular detection of *Babesia caballi* in ticks collected from grazing areas in the state of Rio de Janeiro, Brazil	**Host-parasite-pathogen relationships**
0167	**Joseph Daniel Farrimond:** Kinetics of tick-borne infections in cattle under trypanosomiasis control	
0175	**Monize Gerardi:** Experimental infection of an *Amblyomma sculptum* population with an autochthonous strain of *Rickettsia rickettsii*	
0177	**Patricia Martinez Évora:** Compared acquisition of resistance in domestic dogs to *Rhipicephalus sanguineus* from tropical (Brazil) and temperate (Argentina) regions after successive infestations	
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0218	**Christian Barend Stephanus Hendriks:** Towards understanding the host-vector-pathogen interactions of African swine fever virus in domestic suids	
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0166	**Marylin Hidalgo:** New serological and molecular evidence of spotted fever group rickettsiae in Villeta, Colombia	
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0016	**Marcos Rogério André:** Tick and flea-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil	
0017	**Amélie Chastagner:** Three clusters of *Anaplasma phagocytophilum* isolates from clinical cases of French domestic animals revealed by multi-locus sequence analysis	
0018	**Marcos Rogério André:** Molecular detection of Anaplasma species in dogs in Colombia	
0021	**Awelani Mutshembele:** Epidemiology and evolution of genetic variability of *Anaplasma marginale* in South Africa	
0028	**Francisco Ruiz-Fons:** Dynamics of individual exposure to *Coxiella burnetii* infection in a Q fever endemic red deer (*Cervus elaphus*) farm	
0034	**Adrian Estrada-Sánchez:** Fourier-transformed remote sensing data has superior performance over other abiotic variables to describe the niche of ticks	
0043	**Joon-seok Chae:** A survey of severe fever with thrombocytopenia syndrome (SFTS) virus detection from wild animals and Ixodidae ticks in Korea	
0046	**Joon-seok Chae:** A Survey of Ticks (Acari: Ixodidae) and Severe Fever with Thrombocytopenia Syndrome (SFTS) Virus Infection in National Parks	
0047	**Joon-seok Chae:** Detection of severe fever with thrombocytopenia syndrome (SFTS) virus from domestic pigs in Korea	
0048	**Jeong-Byoung Chae:** Survey on distribution of ticks in domestic pigs, wild boars and their habitats	
0052	**Michal Stanko:** Effect of climatic factors on the occurrence and dominance of ticks in the karst region of Slovakia.	
0055	**Matias Pablo Juan Szabó:** Cattle and ticks in the Brazilian wildlife rich Pantanal: impact on environmental infestation	
0065	**Olivier Sparagano**: The first isolation of *Brucella melitensis* (Rev. 1) vaccine strains from adult small ruminants in Sicily	
0067	**Rivalani Mthombeni:** The development and partial validation of an OpTSGP1 ELISA	
0068	**Algimantas Paulauskas:** Molecular detection and characterization of *Babesia* species in cervids and in ticks infesting cervids in Lithuania	
0070	**Alberto Espí Felgueroso:***Borrelia burgdorferi* S.L. among questing ticks and small mammals in Northern Spain Natural Reserve (Sierra del Sueve-Asturias)	
0099	**Magalie René-Martellet:** Update on epidemiology of canine piroplasmosis in southern France	

0114	**Alessandra Torina:** Ixodidae distribution in relation with climate and environmental factors in the Natural Reserve of Monte Pellegrino in Sicily, Italy	**Epidemiology, ecology and modelling for prevention and prediction**
0115	**Sandor Szekeres:** Ticks and rodents with *Anaplasma phagocytophilum* and Candidatus Neoehrlichia mikurensis infection in Southern Hungary	
0133	**Zuzana Svitálková:** Three-years study of *Babesia* sp. and *Anaplasma phagocytophilum* in questing ticks in Southwestern Slovakia	
0135	**Zuzana Svitálková:** Molecular detection of tick-borne pathogens in rodents and rodent-attached ticks in SW Slovakia.	
0136	**Ana L. Garcia-Perez:** Clinical and laboratorial findings in an outbreak of tick-borne disease in naïve Assaf dairy sheep in northern Spain	
0137	**Bronislava Víchová:** The prevalence and the seasonal dynamics of tick-borne pathogens in questing ticks from the Slovak Karst region, Central Europe.	
0140	**Maria Kazimirova:** Abundance of Ixodes ricinus in an urban and woodland area in South-western Slovakia	
0142	**Maria Kazimirova:** Prevalence of *Candidatus Neoehrlichia mikurensis* in questing ticks and rodents in South-western Slovakia	
0161	**Candice Sant:** Transplacental Transmission of *Babesia caballi* in Thoroughbred Foals in Trinidad	
0164	**Lidia Chitimia:** New date of spatial distribution of *Dermacentor reticulatus* tick in Romania	
0169	**Jesús Alfredo Cortés Vecino:** Some Approaches to the Seasonal Dynamics of *Amblyomma cajennensesensu lato* in Villeta, Colombia	
0183	**Alessandra Torina:** Efficacy of different mosquito trapping methods in a humid area in Sicily	
0187	**Bronislava Víchová:** Ticks and tick-borne pathogens in attractive tourist destinations of Croatia and Greece	
0196	**Jürg M. Grunder:** Automatized analysis of behaviour activity with ticks (*Ixodes ricinus*)	
0201	**Igor Majlath:** Blood parasites of the genus *Hepatozoon* found in snakes from Africa, America and Asia	
0205	**Viktoria Majlathova:** The diversity of hard ticks (Ixodidae) in Slovakia	
0213	**Katia Maria Famadas:**Soft ticks (Argasidae) on frogs *Thoropa miliaris* (Spix, 1824) (Anura: Cycloramphidae) in Brazil.	
0220	**Joon-Seok Chae:** Prevalence of *Anaplasma, Bartonella* and *Borrelia* species in *Haemaphysalis longicornis* collected from goats, Democratic People's Republic of Korea	
0231	**Olivia Mapholi:** Assessments of tick dominance in South African Nguni cattle	
0242	**Sandra Antunes:** What are Portuguese ticks hiding?	
0245	**Maria Margarida Santos-Silva:** Surveillance for ticks and tick-borne pathogens in field and animal populations sharing Iberian lynx habitat	
0250	**Monika Mackiewicz:** Prevalence of tick-borne pathogens in Northern Germany	
0264	**Jumari Steyn:** Epidemiology of bluetongue virus in Mnisi, Mpumalanga	

0009	**Alan R. Walker and Stephen C. Barker:** The character matrix approach to tick identification	**Morphology, systematics and evolution**
0039	**Michael L. Levin:** Phylogeography of the Brown Dog Tick (*Rhipicephalus sanguineus sensu lato*)	
0189	**Diego Garcia Ramirez:** Description of nymphs of *Ornithodoros brasiliensis Aragão*, 1923 (Acari: Argasidae) based on optical and scanning electron microscopy	
0197	**Maristela Peckle Peixoto:** Genetic diversity of *Theileria equi* 18S rRNA gene in horses from Rio de Janeiro, Brazil	
0204	**Katia Famadas:** Free-living ixodid ticks in an urban Atlantic Forest fragment, Brazil	
0207	**Valeria Castilho Onofrio:** New records of Ixodes species collected in the municipalities of Cotia and Itapevi, State of São Paulo, Brazil	
0211	**Diego Garcia Ramirez:** Biology of *Amblyomma calcaratum* Neumann, 1899 (Acari: Ixodidae) in the laboratory	
0217	**Zama Khumalo:** Molecular detection and characterisation of *Anaplasma* species in African buffalo (*Syncerus caffer*) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa	
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0061	**Rashed Ahmed Abdelnabi:** Mosquitocidal activity of a group of essential oils (monoterpenoids) against *Culex pipiens* L (Diptera, Culicidae) In vitro assessment	
0152	**Andreas Turberg:** Inhibition of egg laying and larval hatch in ixodid ticks treated with sub-lethal concentrations of flumethrin and flumethrin/imidacloprid combination	
0259	**Gabriela Aguilar Tipacamú:** Assessment of genetic variability in chloride channel dependant glutamate of a susceptible and resistant strain of *Rhipicephalus microplus* to Ivermectin	
0254	**David Pleydell:** Bayesian prediction of Amblyomma variegatum dynamics using hidden process models	Epidemiology, ecology and modelling for prevention and prediction
0267	**Libor Grubhoffer:** The presence of spirochetes from *Borrelia burgdorferisensu lato* complex and tick-borne encephalitis virus in zoo animals in the Czech Republic	
0176	**Diego Ramirez García:** First report of the isolation and Molecular Characterization of Rickettsia amblyommii from Amblyomma cajennese sensu stricto in Maranhão, northeastern Brazil	Host-parasite-pathogen relationships
0268	**Dieter Heylen:** Transmission dynamics of *Borrelia* bacteria in a bird tick community	
0273	**Hein Stoltsz:** Attempted infection of common waterbuck (*Kobus ellipsiprymnus*) with buffalo-derived *Theileria parva*	
0004	**Jiri Nepereny:** Onset and duration of immunity in dogs after administration of the preparation based on FERRITIN 2 recombinant protein	Immunity and vaccines
0006	**Jiri Nepereny:** Testing of activity of the growth-inhibiting antibodies using in vitro growth inhibition test	
0032	**Philasande Gaven:** Salivary gland transcriptome of female *Rhipicephalus (Boophilus) decoloratus*	
0076	**Olivier Sparagano :** The isolation and identification of potential vaccine antigens against poultry red mite	
0105	**Alessandra Torina:** Effects of recombinant Subolesin vaccine on tick infestations in cattle and sheep farms	
0106	**Pavlina Bartikova:** Ticks with long hypostome induce morphological changes in different cell lines	
0107	**Iveta Stibraniova:** Long mouthparts ticks versus short mouthparts ticks in anti-growth factors activities	
0112	**Lorelle Bizaare:** Characterisation of a lipocalin-like protein from *Ornithodoros savignyi*	
0113	**Iveta Stibraniova:** Effects of immunization with the tick-derived AvPDI protein on feeding and metamorphosis of different tick species and transmission of *Borrelia afzelii* by *Ixodes ricinus*	

0120	**Lucilla Steinaa:** Perforin and CD25 expression in *Theileria parva* specific CTL correlate with cytotoxicity	

0123	**Shivani Goolab:** Evaluating cell surface display as a potential brucellosis antigen delivery system	**Immunity and vaccines**
0127	**Martin Palus:** Changes in global gene expression in brains of mice with different clinical course of tick-borne encephalitis	
0132	**Antoinette Irene Josemans:** The development of live attenuated tissue culture vaccine against heartwater in South Africa	
0148	**Gustavo Seron Sanches:** Effective immunogenic chemical conjugations for P0 antigen in dogs against Brazilian *Rhipicephalus sanguineus* ticks	
0154	**Múcio Flávio Barbosa Ribeiro:** Evaluation in BALB/c mice of the vaccine of *Anaplasma marginale* produced in IDE8 cells associated with rMSP1a, using carbon nanotubes as a carrier molecule.	
0162	**Joaquin Pattarroyo:** Intestinal damages in ticks feeding on immunized cattle with a subunit vaccine against *Rhipicephalus microplus*	
0194	**Robert Miller:** Research project for integrated control of the Southern Cattle Fever Tick in Puerto Rico	
0222	**Mariette Ferreira:**Evaluation of the protective ability of novel antigens against the tick, *Rhipicephalus microplus*, in cattle	
0230	**M. C. Marufu:** Resistance to Rhipicephalus ticks in Nguni cattle reared in the semiarid areas of South Africa	
0233	**Ricardo Pérez-Sánchez:** An RNAi-based approach for screening salivary protective antigens in argasid ticks	
0236	**Tetsuya Tanaka:** The potential of recombinant ferritin 1 and ferritin 2 as anti-tick vaccine against *Haemaphysalis longicornis*	
0238	**Ricardo Pérez-Sánchez:** Assessment of the protective efficiency of the *Ornithodoros moubata* apyrase and savignyigrin orthologues as vaccine targets	
0239	**Ricardo Pérez-Sánchez:***Ornithodoros moubata* salivary secreted phospholipase A2 (PLA2): a new P-selectin antagonist ligand and vaccine target	
0253	**Patricia Martinez Évora:** Immunogenic potential of the recombinant *Rhipicephalus microplus* aquaporin protein against the tick *Rhipicephalus sanguineus* Latreille, 1806 in domestic dogs.	
0270	**Norbert Mencke:** The impact of an imidacloprid/ flumethrin collar on the transmission of *Babesia canis* by *Dermacentor reticulatus* ticks to dogs	
0049	**Siyamcela Genu:** Salivary gland transcriptome of *Rhipicephalus (Boophilus) microplus*	
0271	**Stephen Graves:** Human Pathogens Associated with ticks in Australia	**Zoonoses and public health**
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NOTES:

Poster Session I

Monday 25th August (17h00-19h00)

0025

Recovering Ability of the African Grasscutter (*Thryonomys swinderianus*) to *Trypanosoma* infections

Maxwell Opara, Benjamin Fagbemi
University of Abuja, Abuja/Federal Capital Territory, Nigeria

Objectives

The grasscutter (*Thryonomys swinderianus*) is a wild hystricomorphic rodent widely distributed in the African sub-region and exploited in most areas as a source of animal protein, thus leading to its recent domestication.

Method

Twenty seven (27) captive - reared grasscutters housed in raised iron cages and fed guinea grass (*Panicum maximum*) and water daily, were used to determine the effects of experimental infection of these rodents with *Trypanosoma congolense* and *T. vivax* for 21 days. The PCV, MCHC, total WBC and Lymphocytes of grasscutters experimentally infected with *T. congolense* and *T. vivax* significantly decreased ($p<0.05$), while their MCV significantly increased ($p<0.05$) 21 days post infection (dpi). Plasma glucose and cholesterol were decreased ($p<0.05$). Body temperature fluctuated between 37.4°C and 39.2°C with a peak on day 12 (39.2°C) in *T. congolense* infection and 37.5°C to 40.1°C which peaked on day 8 (40.1°C) in *T. vivax*. The livers and kidneys showed vacuolar and tubular epithelial degenerations respectively, with thrombosis in alveolar blood vessels, but no mortalities.

Conclusions

The results of this study have shown the ability of the grasscutter to harbour trypanosome organisms without any deleterious effect, a factor which could be investigated to understand the reason for the trypano – tolerance. Following this, the grasscutter could thus serve as a candidate animal for vaccine production against African animal trypanosomosis (AAT).

0029

The effects of host and environmental factors on tick parasitism in red deer are modulated by sex

Francisco Ruiz-Fons[1], Pelayo Acevedo[2], Raquel Sobrino[1], Joaquin Vicente[1], Yolanda Fierro[3], Isabel G. Fernández-de-Mera[1]

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Objectives

Host individual, host population and environmental factors interact to modulate parasite abundance in hosts. Since size dimorphism, life history traits and segregation observed in red deer (*Cervus elaphus*) are linked to sex and this ungulate species constitutes a highly relevant host for adult exophilic ticks, it was hypothesized that tick parasitism on stags and hinds would be differentially influenced by each of these factors.

Method

Ticks from 306 red deer were collected during 7 years in a red deer population. By generalized linear models we modelled tick abundance on deer with 20 potential predictors. Three models were developed: one for stags, another for hinds, and one combining data from both sexes and including "sex" as factor. Our rationale was that if tick burdens on males and hinds relate to the explanatory factors in a differential way, it is not possible to precisely and accurately predict the tick burden on one sex using the model fitted on the other sex, or with the model that combines data from both sexes. Our results showed that deer males were the primary target for ticks, the weight of each factor differed between sexes, and each sex specific model was not able to accurately predict burdens on the animals of the other sex.

Conclusions

Results support for sex-biased differences in tick parasitism in red deer. The higher weight of host individual and population factors in the model for stags show that intrinsic deer factors more strongly explain tick burden than environmental host-seeking tick abundance. In contrast, environmental variables better explained tick burdens in hinds.

0040

Anaplasma phagocytophilum strains inhibit apoptosis of *Ixodes* spp. tick cells to enhance early survival and multiplication

Pilar Alberdi[1], Nieves Ayllon[1], M Luisa Martinez de Carnero[1], Lesley Bell-Sakyi[2], Erich Zweygarth[3], Snorre Stuen[4], Jose de la Fuente[1,5]

[1]Instituto de Investigación en Recursos Cinegéticos IREC, Ciudad Real, Spain, [2]The Pirbright Institute, Pirbright, Woking, UK, [3]Ludwig-Maximilians-Universität, Munich, Germany, [4]Norwegian University of Life Sciences, Sandnes, Norway, [5]Oklahoma State University, Stillwater OK, USA

Objectives

Anaplasma phagocytophilum (Ap) is an intracellular rickettsial pathogen transmitted by *Ixodes* spp. ticks, which causes granulocytic anaplasmosis in humans, horses and dogs and tick-borne fever (TBF) in ruminants. In the United States, human granulocytic anaplasmosis (HGA) is highly prevalent while TBF has not been reported. However, in Europe the situation is the opposite, with high prevalence of TBF in sheep and low prevalence of HGA. The origin of these differences has not been identified and is the focus of this research.

Method

In the invertebrate host, Ap multiplies within a parasitophorous vacuole, thus evading host defenses. In this study we used three different strains of Ap of human, canine and ovine origin to infect the *Ixodes ricinus*-derived cell line IRE/CTVM20 and the *Ixodes scapularis*-derived cell line ISE6 to observe the effect of infection on the level of apoptosis in the cells. Inhibition of apoptosis was observed by flow cytometry as early as 24h after infection of both tick cell lines with all three strains of Ap, suggesting that the infection with Ap inhibits the intrinsic apoptosis pathway independently of the origin of Ap strains and vector species.

Conclusions

These results increase our understanding of the mechanisms of Ap infection and multiplication and suggest that other mechanisms affect disease prevalence in different regions.

0051

Experimental infections of HL-60 cells with different strains of *Anaplasma phagocytophilum* isolated from humans, dogs and sheep

Vladimir López[1], Pilar Alberdi[1], Margarita Villar[1], Erich Zweygarth[2], Snorre Stuen[3], Jose de la Fuente[1 4]
[1]Instituto de Investigación en Recursos Cinegéticos IREC, Ciudad Real, Spain, [2]Ludwig-Maximilians-Universität, Munich, Germany, [3]Norwegian University of Life Sciences, Sandnes, Norway, [4]Oklahoma State University, Stillwater OK, USA

Objectives

Anaplasma phagocytophilum is an intracellular rickettsial pathogen transmitted by *Ixodes* spp. ticks, causative agent of granulocytic anaplasmosis in humans, horses and dogs and tick-borne fever (TBF) in ruminants. In the United States, the number of cases of human granulocytic anaplasmosis (HGA) is increasing while TBF has not been reported. In Europe however, the prevalence of HGA is much lower compared to TBF in sheep. The focus of this research is to identify the source of these differences.

Method

In the vertebrate host, *A. phagocytophilum* infects neutrophils and multiplies within a parasitophorous vacuole, thus evading host defenses. Previous research has shown that different strains of *Anaplasma* vary in their ability to infect and cause disease in different vertebrate hosts. Herein, we compared three strains of *A. phagocytophilum* isolated from humans, dogs and sheep, to experimentally infect the human promyelocytic cell line HL-60. Proteomics and transcriptomics analyses demonstrated differences and similarities in the modulation of different signalling pathways, supporting host-pathogen co-evolution.

Conclusions

These results increase our understanding of the infection and pathogenic mechanisms of *A. phagocytophilum* in the vertebrate host and suggest mechanisms refining host-specificity of *A. phagocytophilum* strains that may contribute to the differences observed in disease prevalence in different regions.

0053

Components of tick complement system and their role in the immune response to microbial challenge

Veronika Urbanova[1], Radek Sima[1], Helena Mondekova[2], Tina Flemming[1], Ondrej Hajdusek[1], Petr Kopacek[1]

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Objectives

Ticks are blood-feeding ectoparasites that have a capability to transmit a wide variety of pathogens to their vertebrate hosts. The hard tick *Ixodes ricinus* is the major vector of tick-borne encephalitis virus and spirochetes of the *Borrelia burgdorferi* sensu lato complex in Europe. The transmission of pathogens depends on their ability to evade the tick defense mechanisms, which comprise humoral as well as cellular immune response. Tick innate immunity is based on pathogen recognition and humoral immune reactions that are associated with phagocytosis by tick hemocytes.

Method

Among the immune molecules present in the *I. ricinus* hemolymph, we are mainly focused on fibrinogen-related proteins (FREPs) named ixoderins, complement-like molecules comprising thioester-containing proteins (TEPs) and convertase-like factors (factor C2/Bf and LPS-sensitive Factor C). Here we show the expression profiles for all nine tick TEPs, ixoderins and both convertase-like factors in the response to immune-challenge by different microbes. Functional genomic studies based on RNAi silencing linked with *in vitro* phagocytic assays for various microbes (bacteria, yeasts and spirochetes) by tick hemocytes, allows us to decipher the specific roles that TEPs, ixoderins and convertase-like factors play in the cellular response against these microbes.

Conclusions

Our results on characteristics and function of components of the primordial complement system in ticks contributes to the general knowledge on evolution of this key innate immunity mechanism and offer to design new concepts for efficient blocking of tick-borne pathogens transmission.

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0060

Wild ruminant species as reservoir hosts of tick-borne haemoparasites

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Objectives

Tick-borne diseases (TBDs) of ruminants pose important constraint's to livestock production. Although tick-borne haemoparasites have also been implicated in losses amongst wild animals, the epidemiology and phylogeny of piroplasms of wildlife are largely unknown.

Method

A total of 56 antelope blood samples (eland, waterbuck, impala, buffalo and blue wildebeest) from the National Zoological Gardens and SANParks Biobank's were screened using the reverse line blot (RLB) hybridization assay for the presence of known and novel haemoparasites. DNA was extracted; the V4 hypervariable region of the 18S rRNA gene of *Theileria* and *Babesia* species as well as the V1 hypervariable region of the 16S rRNA gene of *Anaplasma* and *Ehrlichia* species were amplified and subjected to the RLB assay. The RLB results revealed the presence of *Theileria, Babesia, Anaplasma* and *Ehrlichia* species*,* either as single or mixed infections. *Theileria* sp. (sable) (0.12%) and *Theileria ovis* (0.1%) were the most prevalent parasites found (waterbuck and blue wildebeest samples only). Other *Theileria* species present at very low parasitaemia included *Theileria seperata* (0.08%) in waterbuck and blue wildebeest, *Theileria* sp. (kudu) (0.07%) in eland and waterbuck, *Theileria mutans* (0.07%) in buffalo and *Theileria bicornis* (0.05%) in eland and impala. Furthermore *Theileria buffeli*, *Theileria taurotrogi* and *Babesia occultans* were detected.

Conclusions

Importantly, 25% of PCR products failed to hybridize with any species-specific probes, and only hybridized with the *Babesia/Theileria* and/or *Anaplasma/Ehrlichia* group-specific probes suggesting the presence of a novel species or variant of a species within those particular samples. These are currently under investigation.

0064

Transcriptomic analysis of African swine fever virus gene expression during infection

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Objectives

African swine fever is one of the most serious diseases threatening the global pig industry. The disease is caused by the African swine fever virus (ASFV). The virus is transmitted by tick vectors of the *Ornithodoros moubata* complex although transmission via direct contact between animals and fomites can occur. The virus possesses a double-stranded DNA genome consisting of between a 150 and 170 genes. Many of those genes are not required for replication, the rest being involved in host specificity and evasion of host defence mechanisms. While devastating in pigs, infected warthogs and bush pigs remain largely asymptomatic.

Method

We hypothesize that differences in virus replication and the host cellular immune responses are responsible for the reduced susceptibility to disease. It is hoped that identifying these differences would highlight possible targets for vaccine prophylaxis and therapy. However, in order to properly assess the differences between the infection states of pig, warthogs and bush pigs infected with ASFV, a better understanding of the genetic basis of viral replication in the hosts is required. In this study, we sought to characterise the gene expression profile of ASFV during infection of porcine macrophage-monocyte cells. Monocyte macrophages obtained from the lungs, bone marrow and blood of domestic pigs were infected with ASFV. RNA was isolated from the cells at various time points after infection and subjected to Next Generation RNA sequencing and analysis.

Conclusions

It is hoped the results obtained will help illuminate the differences in the host animals' cellular and immunological responses to ASFV infection.

0088

Tick-borne pathogens of camels in Sokoto, Nigeria: Updating some host-pathogen associations

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Objectives

Numerous herds of camels (*Camelus dromedarius*) are reared in northern Nigeria, mostly for meat production. Here, their wellbeing and productivity are constantly challenged by several tick species. At present, however, no information is available on the occurrence of tick-borne pathogens in this livestock species. This study aimed to fill this major gap of knowledge.

Method

In October 2008, whole blood samples were collected from 36 camels (*C. dromedarius*) in Sokoto, North West Nigeria. Collected samples were spotted onto FTA™ cards and, once in the laboratory, subjected to three simultaneous PCRs followed by reverse line blot hybridization (RLB) targeting *Ehrlichia*/*Anaplasma* spp. and *Rickettsia* spp. 16S rDNA and *Theieleria*/*Babesia* spp. 18S rDNA fragments. Following RLB, amplicons were sequenced to ascertain their molecular identity. Twenty-two samples (61%) were positive for *Ehrlichia/Anaplasma*genus-specific probe and three samples (8%) for the *Theileria/Babesia*genus-specificprobe. Three cases of co-infections were also found. All *Ehrlichia/Anaplasma* positive samples were identified as *Anaplasma platys* and all the *Theileria/Babesia* ones as *Theileria ovis*.

Conclusions

To the best of our knowledge, this is the first report of the detection of *A. platys* and *T. ovis* in camels worldwide and in sub-Saharan Africa, respectively. The relevance of this finding is enhanced by the close living of these animals with both dogs and small ruminants, which may also be targeted host species for these pathogens. Results will also be put in relation with the competent tick species (e.g. *Rhipicephalus* spp., *Hyalomma* spp.) potentially acting as vectors for these two microorganisms in the study area.

0091

Global gene expression profiling of virulent and attenuated strains: towards the comprehension of *Ehrlichia ruminantium* pathogenesis

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Objectives

The obligate intracellular bacterium *Ehrlichia ruminantium* is the causal agent of Heartwater, a tropical fatal tick-borne disease in ruminants. Understanding pathogenesis mechanisms of *E. ruminantium* will be helpful for the development of original approaches to control the disease. Global transcriptomic profiling was performed on four *E. ruminantium* strains (Gardel and Senegal strains with both virulent and attenuated phenotypes) using microarray technology. Gardel and Senegal strains are phylogenetically distant and show different behaviour *in vitro*. The microarrays were designed from six sequenced strains genomes (i.e. virulent and attenuated Gardel and Senegal, virulent Welgevonden Erwe and Erwo).

Method

The transcriptomic analysis was done on the extra-cellular infectious form of *E. ruminantium*. The differential gene expression profiling between the samples were correlated with the deletions/mutations present in each virulent and attenuated genomes. Our results showed that there was an over expression of genes coding for metabolic pathway for attenuated strains compared to virulent strains, suggesting their better adaptation to *in vitro* culture conditions. We also observed a main over-expression of *map1*-related genes, coding for outer membrane proteins for virulent strains, whereas attenuated strains over-expressed some genes encoding for hypothetical membrane proteins. The diminution of expression of many genes in attenuated Gardel and Senegal could be linked to severe mutation/deletion events. Moreover, we found the over expression of *LexA* in both attenuated strains. The protein *LexA* is known to negatively regulate the SOS response, a pathway involved in DNA repair. This result suggests a down-regulation of SOS response in attenuated strains compared to virulent strains, thus resulting in higher DNA damage. Moreover, we showed that the gene *recO*, which is involved in DNA repair, harbours a truncation in attenuated Senegal that could explain the higher proportion of mutated genes in attenuated Senegal, inducing the faster attenuation of Senegal compared to Gardel.

Conclusions

Over expression of *map-1* family genes in virulent strains suggests that *in vivo*, MAP-1 family proteins (known to induce non-protective immune responses) could be used by these strains to escape from immune system. In attenuated strains, we observed an over-expression of other membrane proteins encoding genes that could be important to induce protective immune responses, and therefore could be tested as vaccine candidates. This study also highlights that some genes involved in DNA reparation and SOS response could be important in attenuation mechanisms.

0095

Molecular and serological detection of *Theileria equi* and *Babesia caballi* in equids in São Luiz, Maranhão, Brazil

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Objectives

Theileria equi and *Babesia caballi* are tick-borne hemoprotozoan that cause equine piroplasmosis, with a worldwide distribution. The aim of this study was to estimate the occurrence of *T .equi* and *B .caballi* in equids in São Luiz, state of Maranhão, Brazil, by serological and molecular techniques.

Method

EDTA-blood and serum samples were collected from 139 animals (100 donkeys and 39 horses).A crude antigenic preparation of *T. equi*was used to detect antibodies by an Enzyme-linked immunosorbent assay (ELISA). The presence of antibodies to *T.equi* in serum samples was determined by both Indirect Fluorescent Antibody Test (IFAT) and ELISA. Antibodies to *B. caballi* were detected by a recombinant protein (Bc-30 kDa)-ELISA only. Nested PCR assays were based on *ema1* gene (102 bp) for *T.equi* and RAP1 gene (430 pb) for *B. caballi*. Antibodies to *T.equi* were detected in 27.33% (38/139) and 19.42% (27/139) of horses (by IFAT and ELISA, respectively); 32 (25,02%) horses were seropositive to *B. caballi*. Among donkeys, 7,1% (ELISA) and 13.6% (IFAT) were seropositive for *T.equi*; 32 (23,02%) donkeys were seropositive for *B. caballi*. Out of 39 horses, 43.5% and 48.7% were positive for *T.equi* (by ELISA and IFAT, respectively) and 7.7% for *B*. *caballi* (BY ELISA). *Theileria equi* and B. *caballi* DNA were detected in 23% (32/139) and 55.4% (77/139) of equid blood samples, respectively.

Conclusions

Native and recombinant proteins of *T.equi* and *B. caballi*, respectively, proved to be useful tools in seroprevalence studies. In addition, the results showed that *T.equi* and *B. caballi* circulate among donkeys and horses in São Luiz Island, north-eastern Brazil.

0101

Ticks and fleas on small mammals in natural foci of Eastern Slovakia

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Objectives

Rodents and insectivores are hosts for fleas and ticks which may act as vectors of zoonotic and potentially zoonotic microbial pathogens in natural foci. The aim of this study was to investigate the qualitative and quantitative structure of the ectoparasite communities of small mammals in chosen sampling sites, representing peripheral forests and parks in urban and suburban areas of Eastern Slovakia.

Method

A total of 996 individuals belonging to six rodent species and four insectivore species were collected by means of a live and snap traps from 2011 through 2013. Dominant species of small mammals were *Apodemus agrarius* (34.9%), followed by *A. flavicollis* (32.3%), *Clethrionomys glareolus* (27.3%), *Sorex araneus* (1.7%), *and Microtus arvalis* (1.4%). Out of all small mammals trapped at all sampling sites, 53.3% were infested with at least one ectoparasite. 1160 fleas were extracted from small mammals comprising 14 species. Dominant were *Ctenophthalmus agyrtes* (33.1%), *C. solutus* (31.8%), *Megabothris turbidus* (11.9%) and *C. assimilis* (9.8%). About 2290 ticks belonging to *Ixodes ricinus* and *I. trianguliceps* were collected, with a higher prevalence (95%) of the former.

Conclusions

Molecular detection of selected severe pathogens of natural focal infections in tissues of small mammals and their ectoparasites is in process. Preliminary molecular results confirmed circulation of severe pathogens (*Bartonella* spp., *Rickettsia* spp.) in natural foci of urban environment.

The research was supported by Slovak Research and Development Agency APVV 0267-10, VEGA 1/0390/12 and EU project FP7-261504 EDENext.

0104

Experimental infection of domestic pigs with African swine fever virus to investigate transmission cycles

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Objectives

African swine fever is a devastating haemorrhagic viral disease of swine. Transmission of African swine fever virus (ASFV) in South Africa is primarily via the *Ornithodoros* tick-vector. Once in domestic pigs the virus is spread through contact between pigs. As vaccines and treatments are lacking, and the vector cannot be controlled, prevention of the disease relies on strict control measurements. The aim of this study was to investigate, the role of the sylvatic cycle *Ornithodoros* tick in vectoring the disease under experimental conditions.

Method

Pigs were housed in a containment facility, and naturally infected ticks were allowed to feed on domestic pigs, to facilitate viral transmission. An in-contact pig was included to confirm horizontal transmission of the virus from infected to uninfected pigs. ASF clinical symptoms were observed in a pig exposed to infected ticks and transmission of the virus from infected pigs to uninfected ticks was confirmed as was pig to pig transmission.

Conclusions

This is the first study to investigate *Ornithodoros*-facilitated transmission cycles under experimental conditions.

0125

Rickettsia spp. in ticks, rodents and free-ranging ungulates in natural habitats of Southwestern Slovakia

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Objectives

Rickettsia spp. of the spotted fever group are obligate intracellular bacteria transmitted by ixodid ticks. Currently 26 *Rickettsia* species with validated and published names have been reported, some of them are pathogens of humans. This study investigated infections of questing ticks, feeding ticks removed from hosts and infections of hosts with *Rickettsia* spp. in a forest habitat in South-western Slovakia.

Method

In total 2540 *Ixodes ricinus* ticks (2116 nymphs, 424 adults) were collected by blanket-dragging the vegetation from early May 2011 to October 2013. A total of 224 rodents (*Apodemus flavicollis*, *A. sylvaticus*, *Microtus arvalis*, *M. subterraneus*, *and Clethrionomys glareolus*) were live-trapped and 66 spleen samples from *Capreolus capreolus*, *Cervus elaphus*, *Sus scrofa* and *Ovis musimon* were obtained from hunters. One hundred and ninety and 376 *I. ricinus* and *Haemaphysalis concinna* were removed from rodents and ungulates, respectively. *Rickettsia* spp. was detected in extracted DNA using PCR-based methods amplifying partial regions of the *gltA* and 23S rRNA genes. *Rickettsia* spp. was detected in questing ticks, feeding ticks from hosts and in spleen from rodents. Sequencing showed infections with *R. helvetica*, *R. monacensis* and unidentified *Rickettsia* spp.

Conclusions

The results showed circulation of pathogenic species of Rickettsia in Slovakia among *I. ricinus* as a vector and rodents and free-ranging ungulates as transmitters of Rickettsia-positive ticks.

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0130

In vitro establishment of *Anaplasma phagocytophilum* from roe deer (*Capreolus capreolus*) in IDE8 tick cell cultures

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Objectives

Anaplasma phagocytophilum is an emerging pathogen of human and veterinary importance. It is transmitted by Ixodid ticks and it is an obligate, intracytoplasmatic Gram-negative bacterium belonging to the Anaplasmataceae family. It has been described in several animal species, among them roe deer (*Capreolus capreolus*), a wild ruminant species from Europe.

Method

EDTA-blood samples were collected from 75 hunted roe deer from a forest in Southern Germany. White blood cells were isolated for culture initiation. These cells were resuspended in *Anaplasma* culture medium and transferred into IDE8 tick cell cultures. Cultures were kept at 34°C in 25 cm^2 plastic culture flasks. Cell samples were removed, microscopic slide were prepared, stained with Giemsa and examined under a light microscope. Cultured organisms were further analysed by PCR followed by partial sequencing of the 16S rRNA gene. Nineteen IDE8 tick cell cultures became microscopically positive between 64 and 139 days after culture initiation. *A. phagocytophilum* cultures were continuously propagated for at least several passages before cryopreservation. Regular subcultures were carried. Passage intervals varied, depending on the *A. phagocytophilum* strain and the subculture ratio used. Up to now, a partial sequencing of the 16S rRNA gene has been carried out from 14 strains.

Conclusions

Minute amounts of infected blood are necessary to initiate a culture. Up to now, 19 *A. phagocytophilum* isolates derived from roe deer were cultivated in IDE8 tick cell cultures in our laboratory, 14 of which were characterized by partial sequencing of the 16S rRNA gene. The availability of culture-derived organisms will allow a wide range of experiments. Further studies are underway to sequence more genes (*GroEL, msp2, msp4*).

0141

Characterization of *Babesia rossi* genotypes in dogs diagnosed with canine babesiosis

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Objectives

Babesia rossi is a virulent tick-transmitted parasite responsible for causing canine babesiosis in dogs. Canine babesiosis still remains the cause of mortality and morbidity in dogs in South Africa. Preliminary results have suggested that there might be a link between the parasite genotypes and the disease phenotypes. Therefore, the aim of this study was to determine *Babesia rossi* genotypes in dogs diagnosed with canine babesiosis.

Method

Ten blood samples were collected from sick domestic dogs presented at the Onderstepoort Veterinary Academic Hospital. Six dogs were clinically classified as uncomplicated and four as complicated cases. DNA was extracted from the blood samples and *Babesia rossi* infections were confirmed using the reverse line blot hybridization assay. Only 7 *Babesia rossi* genotypes were amplified from 7 blood samples using real-time PCR, followed by sequencing of these samples. Based on the sequence analysis, *Babesia rossi* genotype 28 was identified in 5 dogs and genotypes 19 and 29 were identified in two dogs respectively

Conclusions

Our results are in agreement with previously published findings that *Babesia rossi* genotypes are associated with disease phenotypes in dogs diagnosed with canine babesiosis.

0145

Ultrastructural study of infection of tick cells with the alphavirus Semliki Forest virus

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Objectives

Ticks transmit a variety of pathogenic arboviruses of medical and veterinary importance. In addition, they may harbour, but may not be capable of transmitting, other viruses they acquire from viraemic hosts in the bloodmeal. Examples include the mosquito-borne pathogens Rift Valley fever virus, West Nile virus and Semliki Forest virus (SFV), which have all been isolated from, or detected in, field ticks in Africa. Tick cell and organ culture systems, combined with the availability of virus constructs incorporating fluorescent reporter genes, may help to shed light on the fate of such viruses within the cells and body of the tick.

Method

Here we present light and electron microscopic images of tick cell and organ cultures infected with SFV expressing green fluorescent proteins. Cells of the *Rhipicephalus* (*Boophilus*) *decoloratus* cell line BDE/CTVM16 infected with SFV4(3F)-ZsGreen were processed for transmission electron microscopy (TEM), and organ cultures of developing adult *Rhipicephalus evertsi* were infected superficially with SFV4-steGFP and viewed by incident UV and brightfield microscopy. Over 90% of BDE/CTVM16 cells were infected with SFV at 24 h post infection; TEM revealed numerous alphavirus-like particles budding from the outer membrane of many of the cells, and a few structures resembling components of virus replication complexes were seen in the cytoplasm. SFV infection as determined by presence of green fluorescence was widespread in most tissues of the *R. evertsi* organ cultures at 24-48 h post infection, with the clear exception of midgut cells.

Conclusions

These experiments confirmed the ability of SFV to infect and replicate in many tick cells and organs; further studies are required to determine whether entry into, or exit from, midgut cells may constitute natural barriers to transmission of SFV and other alphaviruses by ticks.

0153

The influence of small mammal host communities on ticks and tick-borne pathogens in Baden-Wuerttemberg, Germany

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Objectives

Ticks are the major vectors of zoonotic pathogens in Central Europe and evidence for an increase in distribution and abundance of ticks and tick-borne pathogens has accumulated over the last decades. To date, however, there are only few comprehensive studies about the factors that influence their distribution and dynamics, particularly those including small mammal hosts.

Method

In four model habitats in Baden-Württemberg, small mammals were trapped from May to October 2012 and March to October 2013 using Longworth live traps. Captured individuals were determined to species, sex, and reproductive state. They were weighed, individually marked and examined for ticks. Ticks were collected, determined to life stage, species, level of engorgement, and subsequently analyzed for the presence of an infection with *Borrelia burgdorferi* sensu lato, *Rickettsia* spp., tick-borne encephalitis virus (TBEV), and *Babesia* spp. The dominant host species at all sites and in both capture seasons were the yellow-necked mouse *Apodemus flavicollis* and the bank vole *Myodes glareolus*. These two species seem to differ distinctively in their relevance as bloodmeal hosts - with *A. flavicollis* carrying up to five times more ticks - as well as in their reservoir capacity with respect to the pathogens mentioned above.

Conclusions

Here, we will analyze the relative effect of small mammal communities on the dynamics of ticks and pathogens in the four selected habitats and their possible future impacts.

0157

Serological evidence for *Anaplasma phagocytophilum* presence in feral horses from Danube delta, Romania

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Objectives

Anaplasma phagocytophilum, the causative agent of granulocytic anaplasmosis, is an emerging pathogen with differential host tropisms and pathogenicity, circulating in wild and domestic animal populations, including horses, transmitted by *Ixodid* ticks. The second largest wetland in Europe, the Danube delta in Romania, is the only delta in the world entirely declared as a Biosphere Reserve, bringing together in the same habitat *Ixodid* ticks and also approximately 1,000 feral horses, around the Letea Forest. This is the first study to evaluate the prevalence of anti-*A. phagocytophilum* antibody distribution in clinically healthy feral horses from the Danube delta in Romania.

Method

Ninety-four equine sera, randomly selected out of 254 samples used to detect equine infectious anaemia during summer season were tested for the presence of anti-*A. phagocytophilum* antibodies by indirect immune fluorescence technique, using MegaScreen FLUO ANAPLASMA ph. (Diagnostik Megacore, Austria). Seroconversion was present in 65.96% (n=62) of the samples with more intense fluorescence than in control sera, while 10.64% (n=10) of the samples showed a lesser, non-specific fluorescence. These results were significantly higher ($p<0.001$) than those obtained in domestic working and sports horses from other regions of the country (8.92%, positive 10 of 112) (unpublished data).

Conclusions

The results indicated that clinically healthy feral horses were naturally infected with *A. phagocytophilum* and more exposed to tick-borne infections in a distinctive habitat such as the Danube delta wetland, than domestic horses located in dryer areas, thus raising the awareness concerning a potential zoonotic cycle.

0158

Natural infection of *Theileria equi* in ticks collected from grazing areas in the state of Rio de Janeiro, Brazil

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Objectives

Equine piroplasmosis caused by *Theileria equi* is considered the major impediment to the international migration of horses. The current study aimed to detect *T. equi* infecting naturally ticks collected from horse pastures in the state of Rio de Janeiro, Brazil.

Method

The collections were performed in 28 properties between January and August 2013. Ticks were collected from horse pastures by dragging flannels over the vegetation and using CO_2 traps. Ticks were identified, counted, separated by life stage and pools of ticks (for each stage and genus) were formed. Genomic DNA was extracted and samples were analyzed by qPCR. A total of 4,716 ticks were collected, generating 414 tick pools: 351 pools were formed by *Amblyomma* genus (199 larva, 144 nymphs, and 8 adults), 38 formed by *Dermacentor* genus (38 larvae) and 25 pools of *Rhipicephalus* ticks (24 larvae, 1 adult). qPCR analysis showed 5.8% (n=24/414) positive tick pools; analyzing individually the tick pools for each genus, it was observed that: 5.1% (n=18/351) *Amblyomma* pools, 13.2% (n=5/38) *Dermacentor* tick pools and 4.0% (n=1/25) *Rhipicephalus* pools amplified the 18SrRNA gene fragment. Interestingly, all 3 genera had positive larvae pools; resulting in 7.3% (n=19/261) larva infected by *T. equi*.

Conclusions

Amblyomma, *Dermacentor* and *Rhipicephalus* ticks may naturally be infected by *T. equi* in the state of Rio de Janeiro, Brazil. Detection of *T. equi* in early stages of ticks (i.e.; larvae) is described for the first time in these tick genera, strongly suggesting the occurrence of transovarial transmission.

0160

Molecular detection of *Babesia caballi* in ticks collected from grazing areas in the state of Rio de Janeiro, Brazil

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Objectives

Equine babesiosis stands out among the diseases that affect the majority of horse herds in the world. This disease is caused by *Babesia caballi*, an intra-erythrocytic protozoan. The present study sought for *B. caballi* parasites naturally infecting ticks collected from horse or cattle pastures in the state of Rio de Janeiro, southeast Brazil.

Method

Collections were performed in 28 properties between January and August 2013. Ticks were collected from pastures by dragging flannels over the vegetation and using CO_2 traps. Ticks were identified, counted, separated by life stage and pools of ticks were formed (for each stage and genus). Genomic DNA was extracted and the samples were analyzed by nested PCR using BC48 fragment as the target. A total of 4,716 ticks were collected, generating 414 pools of ticks: 351 pools of *Amblyomma*, 38 pools of *Dermacentor*, and 25 pools of *Rhipicephalus*. From the 414 pools, 1.9% (n=8/414) were positive for *B. caballi*. Analyzing individually the tick pools for each genus, it was observed that: 1.7% (n=6/351) *Amblyomma* tick pools and 5.3% (n=2/38) *Dermacentor* tick pools had the target fragment amplified.

Conclusions

In conclusion, *Amblyomma* and *Dermacentor* ticks must be naturally infected by *B. caballi* in the region described above. The occurrence of *B. caballi* in *Amblyomma* ticks has important implications for new aspects in the life cycle of this protozoan.

0167

Kinetics of tick-borne infections in cattle under trypanosomiasis control

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Objectives

Together with tick-borne pathogens, animal African trypanosomiasis (AAT) represents a constant threat to cattle fitness and production throughout sub-Saharan Africa (SSA), including Nigeria. In this West African country, a BBSRC-funded intervention programme was recently rolled out as a response to the alarming high prevalence of AAT documented in the indigenous (i.e. *Bos indicus*) cattle from the region. Based on an 11-month long case-control trial, the programme aimed to halt AAT incidence in cattle by controlling the tsetse vector via monthly administrations of deltamethrin-based products.

Method

Under these circumstances, this study aimed to assess the impact of pour-on and spray formulations of deltamethrin on the kinetics of tick-borne infections (TBIs) in indigenous cattle from an endemic area of Nigeria. From March 2012 to February 2013, blood was collected every three months from control and treated cattle in two villages from the Plateau State. Blood samples were spotted on to FTA™ cards and, once in the laboratory, subjected to a molecular protocol consisting of three simultaneous polymerase chain reactions followed by reverse line blot hybridisation targeting up to 25 species within five genera of bovine tick-borne microorganisms including *Anaplasma*, *Ehrlichia*, *Babesia*, *Theileria* and *Rickettsia* spp.

Conclusions

Results will be discussed comparing the treated with control groups and taking into account previous knowledge on the epidemiology of cattle tick-borne infections (TBIs), ixodid fauna and seasonality in the study area. The data generated would serve orientate the devising of sustainable control strategies across SSA, aiming to tackle tsetse-borne trypanosomiasis in TBI-endemic areas.

0175

Experimental infection of an *Amblyomma sculptum* population with an autochthonous strain of *Rickettsia rickettsii*

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Objectives

The pathogen *Rickettsia rickettsii* is transmitted in Latina America mainly by ticks of the *Amblyomma cajennense* complex (including *Amblyomma sculptu*m).

Method

Recently, we isolated a *R. rickettsii* strain (strain ITU) from *A. sculptum* ticks collected in Brazil. Thereafter, we established a laboratory colony of *A. sculptum* from this same locality, and exposed these ticks to its autochthonous strain of *R. rickettsii* (strain ITU). F1 larvae were allowed to feed on febrile guinea pigs that had been needle-inoculated with strain ITU. Subsequent stages were fed on naïve rabbits or guinea pigs until the stage of F2 nymphs. F1 larvae acquired the infection and maintained it to at least the subsequent stage (F2 nymphs), since a rabbit exposed to these nymphs developed clinical signs of rickettsiosis (fever and scrotal reactions) and seroconverted to *R. rickettsii*. However, we were unable to find a *R. rickettsii*-infected unfed nymph, since none of the 20 individuals tested by PCR yielded amplicon. In the subsequent stage, 1/42 (2.4%) unfed F1 adult was PCR-positive; however, adult feeding did not transmit *R. rickettsii* to rabbits. All 19 engorged females and their eggs that were tested by PCR yielded no rickettsial DNA. The F2 larvae did not produce clinical sign on guinea pigs, which did not seroconvert. Finally, all 20 F2 nymphs tested by PCR were negative for rickettsial DNA.

Conclusions

These results confirm previous findings that *A. sculptum* (reported as *A. cajennense*) is partially refractory to *R. rickettsii* infection. Herein, we showed that this refractoriness occurs even for an autochthonous strain.

0177

Compared acquisition of resistance in domestic dogs to *Rhipicephalus sanguineus* from tropical (Brazil) and temperate (Argentina) regions after successive infestations

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Objectives

Comparative studies between *R. sanguineus* populations from Brazil (Jaboticabal) and Argentina (Rafaela) showed marked biological, morphological and genetic differences between them. The present work aimed to study, in a comparative way, acquisition of resistance in domestic dogs to *R. sanguineus* from Jaboticabal (tropical region), and Rafaela (temperate region) after three successive and controlled infestations at 30 days interval.

Method

The ticks were kept in a BOD incubator under controlled conditions, and Dachshund dogs, males and females, aging three months to one year, without prior contact with ticks, were used as hosts. The dogs were distributed into two experimental groups of five animals (n = 5) each, being the first group (G1) infested with ten adult *R. sanguineus* couples from Jaboticabal, per animal, and the second (G2) one with ten adult *R. sanguineus* couples from Rafaela, per animal. Ticks' biological parameters and titration of antibodies from the dogs' sera by ELISA test were used for strains´ comparison. Biological parameters´ results pointed out that dogs do not acquire immunity to both *R. sanguineus* strains after repeated infestations. The ELISA test showed low production of serum antibodies in G2 in successive infestations, and higher production post second and third infestations in G1. It also demonstrated cross-reactivity between sera of dogs infested with *R. sanguineus*, Jaboticabal strain, and antigen from *R. sanguineus*, Rafaela strain, and vice versa.

Conclusions

It can be concluded that there is no significant difference between the acquisition of resistance by domestic dogs to Jaboticabal and Rafaela strains of *R. sanguineus.*

0195

Ticks shape the host adaptive immune response via toll-like receptors

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Objectives

We have shown that tick saliva can modulate cells of the innate immunity, such as dendritic cells (DCs) stimulated with Toll-like receptors (TLRs) ligands. More recently, TLRs have emerged as key sensors also for the adaptive immunity by orchestrating the responses of different lymphocyte populations to diverse microrganisms. The present work evaluates the role of *Rhipicephalus sanguineus* tick infestation in the modulation of the adaptive immune response mediated by TLRs.

Method

We examined the expression of TLRs by flow cytometry on T and B-lymphocytes collected from the lymph nodes of two-time tick-infested mice. We also analysed if TLRs ligands can modulate the lymphocyte immune response after tick-infestation. Finally, the biological parameters of ticks fed on C57/B6 wild-type-(WT) and MyD88-knockout$^{-/-}$ mice, in addition to the host lymphocyte proliferation rate were examined. During tick infestations reduced TLR4 and TLR6 expression on B-cells, and increased TLR5 and TLR9 on T and B-cells, while did not impair TLR1 and TLR2. In addition, tick-infestation decreased T-lymphocyte proliferation when stimulated with TLR1, 4 and 9 ligands; however, these cells showed an activated profile (augmented CD69, CD25, CD86 molecules). Regarding tick-infestation success on MyD88$^{-/-}$ mice, we observed an enhanced number of ticks feeding; egg mass weight, reproductive index and hatching rate. Moreover, tick-infested MyD88$^{-/-}$ mice presented an increase in the lymphocyte proliferation rate when compared with the WT tick-infested mice. Student's t test was used in all experiments (p<0.05).

Conclusions

These data suggest that ticks may use TLRs molecules to shape the adaptive immune response for their success.

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0202

Embryonic cell culture of *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae) for pathogen isolation and cultivation

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Objectives

Ticks transmit a wide variety of pathogens and cause severe diseases such as rickettsiosis, borreliosis, ehrlichiosis and protozoonoses. Bioagents of these two last are transmitted mostly by ticks of the genus *Rhipicephalus*. Some of them doesn´t grow on synthetic culture media. Therefore for isolation and antigen production it is necessary to cultivate them in tick cells. Several lineages tick embryonic cells are already established and used to cultivate pathogens. We herein report a new tick cell culture obtained from, egg masses of *Rhipicephalus sanguineus*.

Method

Eggs from the tick were crushed in L-15B medium, culture kept at 30°C and medium replaced weekly. When a confluent cellular monolayer was obtained they were either frozen in different passages. The cell identity was confirmed identifying a 16S rRNA gene fragment.

Conclusions

Defrosting of cryopreserved cultures was successful.

0208

Investigating the possible presence of *Theileria parva* carrier cattle in Mnisi, Mphumalanga, South Africa

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Objectives

Corridor disease (buffalo-derived *Theileria parva*) caused by *Theileria parva* is the most important *Theileria* sp. posing a threat to the cattle farming industry in South Africa. The African buffalo (*Syncerus caffer*) is the reservoir host for this protozoan parasite transmitted by the three-host ticks *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis*. It is considered a self-limiting disease because most cattle die before parasites reach tick-infective stage. Recent experimental studies have shown that carrier state can be attained in infected cattle that survive the disease. A study to identify *T. parva* carrier cattle in Mnisi, a wildlife/livestock interface area was done (2012-2013).

Method

Records from Hluvukani Animal Health Centre and Bushbuckridge State Veterinary office were scrutinized. Blood samples (n=670) were collected from herds that recorded Corridor disease cases in the past three years, as well as from herds that grazed in areas where buffaloes grazed when they broke out of Kruger National Park. The indirect fluorescent antibody test was used to check for *T. parva* antibodies. Deoxyribonucleic acid (DNA) was extracted from ethylene-diamine-tetra-acetic-acid (EDTA) blood samples collected from sero-positive herds and screened for the presence of piroplasm parasite DNA using a *T. parva*-specific quantitative real-time polymerase chain reaction (qPCR). The p67, p104 and PIM gens were amplified, cloned and sequenced. The sequences are being compared with those found in clinical Corridor disease cases in Mnisi as well as with those previously sequenced from isolates from buffalo.

Conclusions

The IFAT results indicate that there is high prevalence of theileriosis (63.58%) in the Mnisi area although the one associated with *Theileria parva* may be low (13.43%). This may be due to cross reactivity of most *Theileria* species when IFAT is used. Results from samples collected in herds that had clinical Corridor disease cases or sero-positive cattle, show that *T. parva* species-specific qPCR detects piroplasm parasite DNA (2.55%). The *T. parva* parasite transformation or selection from the diverse *Theileria* species in buffalo is therefore of major concern in cattle in Mnisi area.

0218

Towards understanding the host-vector-pathogen interactions of African swine fever virus in domestic suids

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Objectives

African swine fever virus (ASFV) is a double stranded DNA arbovirus that causes a highly contagious disease, African swine fever (ASF), in domestic pigs. Infection results in haemorrhagic symptoms which are fatal to the domestic pig, but not the natural host, i.e. warthog and bush pig. In Sub-Saharan Africa, the disease is enzootic and ASFV persists by means of a sylvatic cycle whereby transmission occurs between the natural host and the disease vector, the argasid tick *Ornithodoros moubata*. It is believed that saliva-assisted transmission is a valid concern and that ASFV infectivity is influenced by salivary components of the tick.

Method

Some aspects that need investigation include the (a) immune system of the domestic pig, which is severely compromised by the immuno-modulatory mechanisms of ASFV, and (b) the role that tick saliva components play in promoting the infectivity of ASFV in the domestic pig. By investigating the transcriptome of ASFV infected domestic pig cells and the transcriptome of ASFV itself through Next Generation Sequencing, we aim to understand the host-vector-virus interactions on a transcriptional level. The expression levels of several host genes have been compared to that of non-infected host cells to investigate transcription regulation by ASFV.

Conclusions

To date, our studies have indicated a number of genes that are differentially expressed and may be candidates for further evaluation.

0235

Effect of salivary gland extract of tick *Ornithodoros porcinus* on African swine fever virus infection in pigs

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Objectives

African Swine Fever is a lethal, hemorrhagic disease of domestic pigs for which animal slaughtering and quarantine are the only control methods available. ASF is historically endemic in Africa and has been recently introduced into Europe. Studies showed the role of *Ornithodoros* ticks in ASFV vector transmission to pigs during blood feeding. However, no study has investigated this interaction during tick bite, especially the role of tick saliva on pig contamination. The pharmacological arsenal of tick saliva is known to modulate inflammatory and immune mechanisms deployed by hosts and it is suspected that such phenomenon exists between ASFV and the soft tick.

Method

A first one-month study was initiated on the impact of salivary gland extracts of *Ornithodoros porcinus domesticus* on the virus infection and the local recruitment of myeloid cells in pigs. We compared the effect of tick bite with intradermal injection of ASFV and intradermal injection of virus added to salivary gland extract on clinical signs, immunological modifications and virus detection in pigs. Cell recruitment at injection site or biting site was also analyzed by immunohistological antibody labelling of cryosections, 1h and 48h post inoculation. Preliminary results indicate a lag in temperature and virological analysis in the group with salivary gland extracts.

Conclusions

This study should showed that gland salivary extracts of ticks tend to modulate innate immune response of pigs, as it has been shown for some other tick-borne diseases. The analyses of cell recruitment, notably of macrophage cells, are in progress and could indicate what the incidence of infection is.

0244

Artificial feeding of *Rhipicephalus microplus* female ticks: effects of serum enriched with anti-calreticulin antibodies on tick and *Babesia bigemina* acquisition

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Objectives

Babesia spp. are tick-borne pathogens that cause a disease called babesiosis in a wide range of animals. Particularity, *B. bovis* and *B. bigemina* are transmitted by cattle ticks, *Rhipicephalus annulatus* and *R. microplus* being considered the most important cattle ectoparasites with major economic impact on cattle production. The use of chemicals to control ticks has drawbacks supporting the need to pursue alternative methods. Anti-tick vaccines are considered a cost-effective and environmentally safe strategy but are still scarce mainly due to the lack of effective antigens. Calreticulin has been identified as being involved in *B. bigemina* infection in *R. annulatus* ticks.

Method

Herein, *R. microplus* females were successfully artificially fed using capillary tubes, a low cost alternative to test antigens allowing achieve critical data. Additionally, recombinant calreticulin (rCRT) protein was expressed in an *E. coli* expression system and antibodies were raised against rCRT. Anti-rCRT serum was supplemented to a blood meal, offered to partially engorged *R. microplus* females and their effect in feeding process as well as infection by *B. bigemina* was analyzed.

Conclusions

No significant reductions in tick and egg weight were observed when ticks fed with anti-rCRT serum. Also, *B. bigemina* infection levels did not show a statistically significant decrease when ticks fed with anti-rCRT antibodies. In spite of these results artificial capillary feeding can be a useful technique for the characterization of candidate tick protective antigens.

0246

Artificial feeding of *Rhipicephalus microplus* female ticks: effects of anti-TROSPA antibodies on tick and *Babesia bigemina* acquisition

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Objectives

Ticks are obligate haematophagous ectoparasites that can transmit a wide variety of pathogens, being considered the principal vectors of disease among animals. The piroplasms *Babesia bigemina* and *B. bovis* are transmitted mainly by *Rhipicephalus microplus* and *R. annulatus* ticks. Anti-tick vaccines despite promising are still scarce mainly due to the lack of effective antigens. Tick proteins involved in tick-pathogen interactions may provide good candidate protective antigens for these new vaccines but their identification and validation are still limiting steps. Appropriate screening procedures are needed to select the best candidates.

Method

In a previous study functional analysis by RNA interference showed that, knockdown of *trospa* significantly reduced *B. bigemina* infection levels in *R. annulatus* and *R. microplus* when compared to controls. Herein, recombinant TROSPA was obtained using *Escherichia coli* expression system and poly/monoclonal antibodies were generated. Their specificity against tick recombinant protein was confirmed by Western blotting and against native proteins in tick tissues using immunofluorescence. Moreover, *R. microplus* females were successfully artificially fed using capillary tubes. Capillary-fed ticks ingested antibodies added to the blood meal and the effect of these antibodies on tick weight and/or oviposition was analyzed.

Conclusions

No significant effect was observed on pathogen acquisition. The results highlight the advantages and disadvantages of *in vitro* tick capillary feeding for the characterization of candidate tick protective antigens.

0258

Antibodies to HAP2 conserved, B cell epitopes, identify *Babesia bigemina* tick stages

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Objectives

Sexual reproduction is a process involving recognition and fusion of two cells and is highly conserved among different taxa. The *hap2* gene, which codifies for a male specific transmembrane protein has been found to participate in gamete fusion in various organisms including plants and protozoa. We have previously identified a homologue of *hap2* in the genome of *Babesia bigemina* that is transcribed and expressed in tick stages. Alignment of HAP2 from six isolates from different parts of the world showed a 98% similarity at the amino acid level.

Method

The aim of this work was to identify conserved, B cell epitopes in HAP2 of *Babesia bigemina*. The amino acid sequence of HAP2 of the Chiapas-Mexico strain was analysed and used to predict hydrophilic and antigenic peptides containing B cell epitopes in the extracellular domain by bioinformatics tools. Four peptides were selected on fully conserved predicted B cell epitopes. Each peptide was chemically synthesized as a dendrimer and used to immunize rabbits. Two rabbits were immunized subcutaneously every two weeks a minimum of three times with 100µg of each peptide emulsified with a water-in-oil commercial adjuvant. A sample of *B. bigemina* infected erythrocytes was used to purify tick stages using a previously described protocol. Rabbit serum samples were used in an immunofluorescence assay on tick stages of *B. bigemina*, and detected with a secondary anti rabbit IgG antibody conjugated with Alexa-488 and analysed by fluorescence microscopy.

Conclusions

All four peptides generated antibodies recognizing the conserved, surface exposed B cell epitope on native HAP2 of *B. bigemina* tick stages. These antibodies can now be used to block sexual stage fusion and tick transmission.

0265

Identification and sequence characterization of novel *Theileria* genotypes from the waterbuck (*Kobus defassa*) in a *Theileria parva*-endemic area in Kenya

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Objectives

Waterbuck are infested by large numbers of *Rhipicephalus appendiculatus*, the tick vector for *Theileria parva*, and previous data suggests that the species may be a source of *T. parva* transmission to cattle. However, there is no evidence that *T. parva* is transmissible to the waterbuck under field conditions. In addition, though the occurrence of Theileria spp. in the waterbuck has been reported, the tick vectors and genotype of these parasites are currently unknown.

Method

A total of 86 cattle and 26 waterbuck blood samples were obtained from Marula, a site in Kenya endemic for East Coast fever where the primary wildlife reservoir of *T. parva*, the Cape buffalo, is also common. A nested PCR specific to *T. parva* p104 gene, and a reverse line blot (RLB) incorporating 13 oligonucleotide probes including all of the available Theileria spp. so far described from livestock and wildlife in Kenya were used to investigate for the presence of cattle-infective *Theileria* parasites. Neither assay provided evidence of *T. parva* or *Theileria* sp. (buffalo) infection in the waterbuck DNA samples. A generic probe for Theileria spp. hybridized with 25/26 of the waterbuck samples while none of the 11 species-specific probes hybridized with the waterbuck-derived PCR products. Phylogenetic analysis of the 18S rRNA and ITS sequences within the RLB-positive waterbuck samples revealed the occurrence of three Theileria genotypes of unknown identity designated as A, B and C. Group A clustered with *Theileria equi*, a pathogenic Theileria species infective to domestic equids. Group B parasites clustered closely with *Theileria luwenshuni* while Group C was closely related to *Theileria ovis*.

Conclusions

The data indicate that waterbuck may not play a role in the epidemiology of ECF at this site since *T. parva* could not be detected in the 26 animals sampled. However waterbuck is a host to multiple additional Theileria genotypes that are genetically related to species that are pathogenic in both small ruminants and equids. The veterinary significance of this observation requires further investigation.

0094

ITS2 markers for molecular taxonomy of Brazilian Ixodidae ticks recognized as vectors of rickettsial agents

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Objectives

The importance of ticks lies not only in spoliative action, but also due the great ability to act as vectors of several pathogens. Traditionally, the identification of ticks is performed based on external morphology, being a process extremely laborious, time consuming, dubious and dependent on skilled personnel, with limitations as to its achievability, depending of the conservation condition and the stage of specimen development. In this sense, was used a molecular strategy for the taxonomic identification, seeking markers that can assist to identify and distinguish different genera and/or species, by amplification and sequencing of internal transcribed spacers (ITS2) markers.

Method

With the aid of multiple alignments of sequences from GenBank we performed the design of specific primers and protocols for standardization of PCR directed at some of the most important ixodidae species in Brazil, especially some related to epidemiology of rickettsial diseases. Three sets of primers allowed the taxonomic identification of the genera *Demacentor*, *Rhipicephalus* and *Amblyomma*. In tests using species-specific primers was successfully obtained for three sets of primers, which confirmed the identification of the efficacy of three species of *Amblyomma*, resulting in amplicons of 405 bp, 503 bp and 321 bp characteristic for species *A. cajennense*, *A. oblongoguttatum* and *A. parvum*, respectively.

Conclusions

It was observed that ITS2 regions are useful for molecular identification of Ixodidae ticks, showing low intraspecies variability. It can be useful to improve the study of tick ecology, epidemiological studies and natural history of ticks and ticks-borne pathogens.

0102

De novo transcriptome assembly of *Rhipicephalus appendiculatus* salivary glands

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Objectives

Ticks are haematophagous arthropod vectors of a variety of human and animal diseases and are well adapted to feed unnoticed on their hosts. Numerous proteins are expressed in the salivary glands and injected into the host to create a stable feeding environment. These proteins modulate the host's haemostasis, inflammation and immune response and are attractive candidates for recombinant anti-tick vaccines. Many of these proteins are uncharacterised due to annotation challenges where searchable sequence databases are small or incomplete. Advances in next generation sequencing technologies have made sequencing of non-model organisms such as arthropods affordable and will expand public sequencing databases.

Method

To this end, we sequenced the expressed genes in the salivary glands of *Rhipicephalus appendiculatus* using the Illumina® HiSeq2000. More than 300 million Illumina paired end sequences were generated and assembled using the Trinity software package. The nearly 60 000 assembled transcripts were filtered based on abundance estimations, reducing the number of expressed transcripts to about 20 000. Open reading frames were predicted using the TransDecoder software and differential expression estimated by RSEM and edgeR/Bioconductor packages. Annotation was based on BLAST alignments against the non-redundant sequence database (Nr), UniProt Knowledgebase (UniProtKB) and Conserved Domains Database (CDD).

Conclusions

The assembled *R. appendiculatus* transcriptome contains known tick salivary gland proteins, highlights expanded protein families and reveals insights into the metabolic pathways involved in blood feeding. By understanding the mechanism of blood feeding behaviour, we will enhance our capability to select vaccine targets.

0146

cDNA library of argasid ticks: high potential for production of diagnostic kits and new drugs

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Objectives

In order to study the biological activity produced by the neotropical argasid ticks *Ornithodoros mimon* and *Ornithodoros brasiliensis*, with potential application in the pharmaceutical industry and tick control.

Method

The cDNA libraries of their organs have been produced. As results, the transcripts present in the gut of *O. mimon* and in the salivary glands of *O. brasiliensis* have shown, respectively, functional activities such as adhesion, transport and catalytic activity, and lipocalins, moubatins and metalloproteases. For *O. mimon*, the catalytic activity and transport/channels are the most representative categories comprising 25% and 14% of the contigs, respectively. Several peptidases associated with the digestion of hemoglobin were also identified representing potential targets for the development of strategies for tick control. For *O. brasiliensis*, the results show that 20.93% of the contigs were categorized as belonging to the secretory pathway and 14.77% as membrane proteins, confirming the glandular tissue. The lipocalins represented 15.6%, while the moubatins, which may be considered as subclasses of lipocalins, were 6.23%. Proteins with acidic tail were 1.63%, and the metalloproteinases added 1.23% of the transcripts. The entire assembly has a role at the control of coagulation activity, in modulating the immune response, in inhibiting platelet aggregation and still acts in the fibrinolytic, and fibrinogenolytic activities.

Conclusions

Those substances produced by these argasids have high potential for production of diagnostic kits and also for the development of new drugs.

0180

Identification, expression and characterisation of *Rhipicephalus microplus* serine protease inhibitors (serpins)

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Objectives

The cattle tick, *Rhipicephalus microplus* causes the most significant loss for tropical and sub-tropical beef industries worldwide estimated at $US2.5b annually. Members of the serine protease inhibitor (serpin) family play important roles during the interaction between hosts and ticks. Tick serpins manipulate the hosts' coagulation system for successful blood feeding, disrupt the hosts' inflammatory and immune responses, and are also involved in tick physiological regulations. Studies of *R. microplus* serpins are limited and thus this study aimed to identify, express and determine the biological activity of the recombinant *R. microplus* serpins.

Method

CattleTickBase was mined for serpin sequences and 13 serpins were identified, named RMS-1 to RMS-14. Seven full length serpins were selected for transcriptional analyses and semi-quantitative RT-PCR demonstrated differential expression in different female adult organs as well as during different tick life stages. The biological function was examined using the recombinant *R. microplus* serpins (rRMSs) expressed by the yeast *Pichia pastoris*. rRMS-4 prolonged blood clotting in an RCT assay and specifically inhibited Thrombin at the inhibition rate of $6.0\pm0.35x10^{4}M^{-1}s^{-1}$. rRMS-3 inhibited Chymotrypsin and Elastase. Chymotrypsin was also inhibited by rRMS-9. ELISA screening demonstrated that rRMS-3 and -4 were recognised by sera collected from tick exposed cattle. To further understand the mechanism of serpin inhibition, the tertiary structure of cleaved rRMS-9 was resolved by crystallisation and X-ray diffraction confirming that RMS-9 undergoes the serpin signature conformational change.

Conclusions

This preliminary study demonstrates the function of *R. microplus* serpins and provides potential avenues in the development of innovative tick control methods.

0188

Identification and characterization of RmSEI, a Protein Presents in the Gut of the Tick *Rhipicephalus microplus*

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Objectives

Ticks are important ectoparasites that transmit a wide range of infectious agents, including arboviruses, rickettsiae, spirochetes, and protozoa, which cause diseases in humans and domestic animals. The *Rhipicephalus microplus* is an exclusive ectoparasite of cattle, being responsible for transmission of *Anaplasma* sp and *Babesia* sp. Our understanding of the protein arsenal in the tick gut is fragmented.

Method

The aim of this work was to confirm the function of RmSEI. To obtain the RmSEI transcription profile in the tick, total RNA extracted from different tissues from engorged females was analyzed by PCR. This analysis showed that RmSEI mRNA was transcribed in all tissues. To elucidate the function of this protein, a cDNA library of *R. microplus* gut was constructed and sequenced. After analysis, we observed a high frequency of transcripts encoding proteins similar to RmSEI (ABH10604.1), suggesting a possible role of these proteins in this organ. Recombinant RmSEI was produced through cloning in pET26b vector and expression in *E. coli* BL21 (DE3) pLysS strain. Purified rRmSEI was tested upon different serine proteases showing no activity for any of them. In order to identify a role for RmSEI, we performed RmSEI gene silencing by RNA interference. Gut and ovary were collected at 24, 42 and 72 hours after dsRNA injections to verify gene silencing in comparison with control groups. Our preliminary results showed an RmSEI expression reduction in 72 h in gut. We also identify an increase in the bacterial community in 24 h, and a reduction in 72 hours using ribosomal primers 16S by PCR.

Conclusions

As perspectives, antimicrobial assays will be performed using purified rRmSEI, and gene expression analysis in different tissues will be conducted through quantitative real time PCR, and understand the possible role of RmSEI in modulating the tick gut microbiota.

0200

Presence of enzymes and anticoagulant action in secretions of three tick species

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Objectives

Proteins isolated from the saliva and other secretions of hematophagous are useful tolls for the understanding of many physiological and evolutionary processes. In this study we propose to investigate the presence of inhibitors and enzymes produced in saliva, hemolymph and coxal fluid of the ticks *Amblyomma sculptum*, *Ornithodoros brasiliensis* and *O. rostratus*; establish the participation of it in these ticks feeding process and identify the active molecules.

Method

The profile protein of saliva, hemolymph and coxal fluid was analyzed by SDS-PAGE. The inhibitory activities were tested on the FXa by amidolitic activity using chromogenic substrate S-2765 by hemolymph and coxal fluid of *O. brasiliensis*. And α-glucosidase activity, β-glucosidase, trehalase and maltase, xylanase and amylase were tested by *A. sculptum* saliva and coxal liquid of *O. rostratus.* The protein profile of the samples shows less than 100 kDa. The haemolymph and coxal fluid showed inhibitory activity on FXa. The presence of the enzymes amylase, maltase trehalase, α-glucosidase, β-glucosidase, and β-galactosidase was observed and lower activities of endoglucanase and xylanase in the saliva of *A. sculptum* and coxal fluid of the tick *O. rostratus.*

Conclusions

The discovery of these molecules may have therapeutically applications, biotechnological advantages in the area of food production and in the optimization of obtaining energy from biofuels.

0206

Two novel cystatins identified in gut and ovary of *Rhipicephalus microplus* - functional and structural studies

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Objectives

The *Rhipicephalus (Boophilus) microplus* is an exclusive bovine ectoparasite responsible for transmission of several pathogens. Tick control is still a challenge, an alternative method has been vaccine production but the main problem is the identification of potential protein targets. Considering that, proteases and their inhibitors play an important role in tick physiological events; they are candidates to be used in immunization experiments.

Method

Recently, a gut transcriptome of *R. microplus* was generated by our group, and one cystatin-coding gene, called *Bm*cystatin4 was identified. In parallel using ovary cDNA of *R. microplus* another cistatin gene called *Bm*cystatin8 was identified. The aim of this work was to confirm the inhibitory activity of both cistatins for cisteine proteases and identification of their physiological function in the ectoparasite. The *Bm*cystatin4 and *Bm*cystatin8 were cloned in pPICZαB and pET28a vectors, and expressed in *Pichia pastoris* - GS115 strain and *E. coli* BL21 -(DE3)-pLysS strain, respectively. Both, rBmCystatin4 and rBmCystain8 were recovered after anionic and nickel affinity chromatography in active forms. Folding studies of rBmcystatin4 and rBmcystatin8 at different temperatures were carried out using circular dichroism (CD). Purified rBMcystatin4 inhibited cathepsin L, cathepsin B, papain and BmCl1, in contrast rBmcystatin8 only inhibited papain. BmCystatin4 expression analysis revealed high expression in the gut by qPCR and Bmcystatin8 seems to be expressed mainly in ovaries as shown by PCR.

Conclusions

As perspectives, Bmcystatin4 and Bmcystatin8 three-dimensional structures are being carried out.

0229

OAKS: optimization and automation of artificial tick feeding

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Objectives

Since ticks require blood for their development, the use of experimental animals is often inevitable in tick and tick-borne disease research. An artificial feeding method for ticks could replace the use of experimental animals, or lead to a reduction thereof. However, all methods developed thus far result in poor feeding- and reproduction ratios in comparison to ixodid ticks fed on animals. They are also laborious, which has hampered the adaptation of these techniques. In this project, critical steps in the artificial tick feeding process are investigated in more detail.

Method

Existing artificial tick feeding systems are evaluated concerning their performance for feeding five ixodid tick species which differ in their mouthpart length, life cycle, host finding strategy, reproduction strategy or host specificity. The influence of parameters relevant to the attachment and feeding process such as membrane composition, atmospheric conditions and blood composition/additives will be examined and their influence on tick feeding success will be statistically validated. Research applications as well as methods to simplify and automate the artificial feeding are also under scrutiny.

Conclusions

Results from the OAKS-project (funding period: 2013-2016) will give insight into factors critical in the artificial feeding of ixodid ticks, with the aim of making this technique more accessible and attractive for scientists working on ticks and tick-borne pathogens.

0073

TBEV along the coast of Southern and Western Norway Andreassen

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Objectives

Tick-borne encephalitis virus (TBEV), a member of the Flaviviridae family, infecting the central nervous system. TBE is endemic in many European countries, and the prevalence has increased the past three decades in Europe as well as in Norway. The knowledge of distribution and prevalence of TBEV in ticks in Norway is limited. The aims of these studies were to estimate the distribution and the prevalence of TBEV along the coast of Southern and Western Norway. We wanted to compare the prevalence between the different areas to see whether these corresponded with the Norwegian government advices for vaccination against TBE.

Method

IgG antibodies to TBEV was examined in sera from human blood donors and wild animals from potential risk areas in Norway. In addition, ticks were collected at different locations according to registrations of human cases of TBE and from nearby municipalities. The tick nymphs were collected between 2009 -2013 and analyzed by real-time PCR.

Conclusions

The preliminary findings may indicate that TBEV is distributed over a larger area in Norway than previously suspected based on the registration of TBE cases.

0103

Bacterial population analysis in tick salivary glands using 16S rDNA amplicon analysis

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Objectives

Ticks are one of the most important blood-sucking vectors of infectious agents. They inject saliva into the host in order to facilitate the uptake of blood. Microorganisms such as Rickettsia in salivary glands are transmitted together with the saliva. An understanding of the microbial populations within tick salivary glands would provide fundamental information on the vector capacity of ticks. The aim of this study was to analyze bacterial populations in tick salivary glands using 16S rDNA amplicon analysis.

Method

Three tick species (*Ixodes ovatus*, *I. persulcatus* and *Haemaphysalis flava*) were collected in Shizuoka Prefecture of Japan. Salivary glands were taken out from each tick. 16S rDNA amplicon pyrosequencing was performed with the Roche 454 GS junior platform. Sequence reads were classified at the genus level using a public database. This study identified a total of 163 different bacterial genera, including the ones of tick-borne pathogens such as Ehrlichia and Rickettsia. In addition, we also detected previously uncharacterized Alphaproteobacteria symbionts in certain tick species. When compared with a conventional PCR assay, this pyrosequencing approach had higher sensitivity in the detection of rickettsial sequences. The principal component analysis revealed that the bacterial populations were different between tick species.

Conclusions

Tick salivary glands contained a variety of bacterial genera. Our strategy makes it feasible to detect both known and as-yet unknown pathogens and therefore is useful for the surveillance of tick-borne pathogens.

0117

The incidence of Murine herpesvirus 68 in wild ticks *Dermacentor reticulatus* occurring in territory of Slovakia

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Objectives

Murine herpesvirus 68 (MHV-68) originally isolated from free-living murid rodent *Myodes glareolus* serves nowadays as an animal model to study human gammaherpesviruses. Recent finding of MHV-68 presence in *Ixodes ricinus* larvae and nymphs removed from some green lizards *Lacerta viridis* opened the issue of its spread in nature including a risk of natural tick-borne transmission of this virus to humans. The aim of study was to find out whether MHV-68 is present in *Dermacentor reticulatus* ticks occurring also in Central Europe.

Method

The group of 312 ticks (females and males) was tested by single copy sensitive PCR specific for ORF50 known as a conserved gene. MHV-68 incidence reached 9.7% and 66% in ticks trapped in west Slovakia in autumn 2011 and spring 2012, respectively. We confirmed the specificity of virus detection by RFLP and sequence analyses of PCR products.

Conclusions

Female ticks were 1.4 times more infected than male ticks regardless of trapping season. Two ticks were found positive for MHV-72, the strain isolated in 1980 from the same rodent species, using RFLP analyses of ORFM3 specific PCR products (carrying specific restriction site), thus proving that MHV-72 is still circulating in nature.

Acknowledgements: This work was supported by the grants #2/0091/13 and # APVV-0621-12.

0155

Anaplasma phagocytophilum in a Brazilian urban dog: clinical, parasitological, hematologic, serological and molecular diagnostics

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Objectives

Anaplasma phagocytophilum is a tick borne pathogen found in North America, Europe and Asia. In Brazil, it has recently been described in animals using molecular and serological methods. The aim of this study was to provide clinical, parasitological, haematological, serological and molecular evidence of a Brazilian dog naturally infected with *A. phagocytophilum.*

Method

A male French bulldog, eight month old, had veterinary clinical examinations in April, June and December 2013. Biochemical and hematological analysis were performed and blood samples were taken. A nested PCR (nPCR) and the indirect immunofluorescent antibody (IFA) test were performed. Morulae were present within neutrophils in blood smears prepared in June. The IFA showed Anti-*A. phagocytophilum* and Anti- *E. canis* IgG were present in the samples taken in June and December. During this time, samples also tested positive for *A. phagocytophilum* and *Ehrlichia* spp. DNA using the nPCR. Subsequent phylogenetic analysis has shown that sequences obtained in this study were closely related to previously described *A. phagocytophilum* sequences, including isolates from dogs and humans. The *Ehrlichia* sequences obtained showed close similarity with *E. canis* isolates. Lethargy was the only clinical signs noted in the dog during the study period. Severe thrombocytopenia has been observed during all examinations. Erythocytes, hemoglobin and hematocrit showed normal values only in December. There was also an increase in alanine aminotransferase before treatment with doxyciclin.

Conclusions

The present work provides a first report of clinical, parasitological, serological and molecular evidence of *A. phagocytophilum* in a Brazilian dog, highlighting the growing importance of this agent in South America.

0165

Simultaneous detection of veterinary and zoonotic pathogens in Nigerian ticks

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Objectives

Ticks and tick-borne pathogens represent a persistent threat to the health of livestock and their keepers throughout sub-Saharan Africa, including Nigeria. The present study aimed to assess the occurrence of both veterinary and zoonotic pathogens in ticks collected from cattle in an area of central Nigeria, where traditional manual removal is the only method employed for ectoparasite control.

Method

Ticks were collected in October 2010 from indigenous (*Bos indicus*) cattle in Plateau State. Collected ticks were preserved in 70% ethanol and morphologically identified. For each tick, sex and feeding state were also recorded. After DNA extraction, all samples were subjected to a molecular protocol consisting of three simultaneous polymerase chain reactions followed by reverse line blot hybridisation targeting *Ehrlichia/Anaplasma* spp. and *Rickettsia* spp. 16SrDNA and *Theileria/Babesia* spp. 18S rDNA. Amplicon identity was then confirmed via sequence analysis. 272 adult ixodid ticks were identified, including 104 *Rhipicephalus annulatus*, 87 *R. decoloratus*, 12 *R. geigyi*, 34 *Hyalomma truncatum* and 35 *Amblyomma variegatum*. Pathogens detected included *Anaplasma marginale, A. bovis, A. centrale, Ehrlichia canis, E. ruminantium, Anaplasma sp. Omatjenne, Theileria mutans, Babesia bigemina* and *Rickettsia* spp.A high frequency of co-infection with *A. marginale* and *Rickettsia* spp. was also seen. Amongst the 34 ticks that tested positive for *Rickettsia* spp., 32% (n=11) tested positive for zoonotic pathogens, such as *Rickettsia africae* (i.e. *A. variegatum, R. annulatus*) *Rickettsia massiliae* (i.e. *R. annulatus, H. truncatum*) and *Coxiella burnetii* (i.e. *A. variegatum, R. decoloratus and H. truncatum*).

Conclusions

Our findings suggest high risk for both cattle and human health.

0166

New serological and molecular evidence of spotted fever group rickettsiae in Villeta, Colombia

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Objectives

Although the town of Villeta is endemic for Spotted Fever Group (SFG) rickettsioses, no new cases of *Rickettsia rickettsii* infection have been reported after 2004. Active surveillance of *Amblyomma cajennense* ticks, its vector, has also failed to detect *R. rickettsii*.

Method

From November to December 2011, we collected 254 (n= 74 horses, n= 118 dogs and n= 62 cattle) serum samples for antibody titer determination against SFG rickettsiae (*R. rickettsii* antigen) using Indirect Fluorescence Assay (IFA, IgG). We also collected 516 ticks in the same period. Ticks were grouped into pools for DNA extraction and tested for rickettsial infection by PCR targeting the rickettsial *ompA* and *ompB* genes. Seropositivity was observed in 33.7% of horses, 14.4% of dogs and 50% of cattle. A total of 6 tick pools were positive for at least one of the two rickettsial genes. BLAST analysis of the sequences of the amplicons revealed identity with *Rickettsia* sp. closely related to *R. conorii* and *R. rickettsii* in *A. cajennense* sensu lato; *Rickettsia* sp. closely related to *R. monacensis* and `*Candidatus* R. amblyommii´ in *Rhipicephalus* (*Boophilus*) *microplus*; and *R. rickettsii* in *Dermacentor nitens* and *Amblyomma* sp.

Conclusions

Herein, we provide the first molecular evidence of the circulation of *R. rickettsii* from *A. cajennense* s.l. in Colombia. In addition we showed evidence of the circulation of at least 4 different SFG rickettsiae, and we also confirmed serological patterns previously observed in domestic animals.

0173

Circulation of *Candidatus, Neoehrlichia mikurensis* and *Babesia microti* among ticks and rodents in natural foci of Slovakia (Central Europe)

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Objectives

Rodents are important reservoir hosts of many tick-borne pathogens. Their importance in the circulation of human pathogens *Neoehrlichia mikurensis* and intraerythrocytic protozoan parasite, *Babesia microti* has been recently proposed. The aim was to identify the presence and genetic diversity of *N. mikurensis* and *B. microti* circulating in the natural foci between the rodents and *Ixodes* spp. ticks and to study their ecological association.

Method

In 2011-2013, rodents were captured on sampling sites in Eastern Slovakia. Biopsies, feeding ticks from rodents and questing ticks were investigated for the presence of pathogens by molecular methods followed by DNA sequencing. *Neoehrlichia mikurensis* was detected in questing *I. ricinus* ticks, biopsies of rodents, as well as in feeding *Ixodes* spp. ticks from rodents. The 16S rRNA and gltA sequences of *N. mikurensis* obtained in this study confirmed high degree of homology. DNA of *B. microti* was found in biopsies of rodents, feeding and questing *I. ricinus* ticks. None of the 109 *I. trianguliceps* ticks was infected with *B. microti*. BLAST analysis of *B. microti* nucleotide sequences confirmed the presence of two genotypes,"Jena strain" (90%) and "Munich strain" (10%).

Conclusions

Results of our study confirmed the importance of rodents in the circulation of both emerging pathogens in the natural foci. This might pose a potential threat for humans.

The research was supported by the Research and Development Operational Programme funded by the ERDF (code ITMS: 26220220116), VEGA 2/0055/11, VEGA 2/0113/12, the Slovak Research and Development Agency under contract No. APVV-0267-10, EU project FP7-261504 EDENext.

0209

Serosurvey of small mammals to spotted fever group *Rickettsia* in the state of Rio Grande do Sul, southern Brazil

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Objectives

Rio Grande do Sul is composed by two biomes, the Atlantic forest in the northern half, the Pampa in the southern half. Cerro Largo municipality, located in a transition area of these two biomes, has been considered a spotted fever-endemic area since 2005. While the *Rickettsia* species involved in human cases has never been confirmed, recent surveys have suggested *Rickettsia parkeri* or a close-related species, vectored by *Amblyomma* species that use small mammals as hosts for immature ticks.

Method

Therefore, from July 2013 to January 2014, small mammals were trapped in (i) Cerro Largo; (ii) one natural Reserve of Atlantic forest in the north; and (iii) one natural Reserve of Pampa in the south. A total of 40 animals of 4 species (*Akodon montensis, Didelphis albiventris, Oligoryzomys nigripes, Sooretamys angouya*) were sampled in Cerro Largo; 164 animals of 8 species (*A. montensis, Brucepattersonius iheringi, Cryptonanus guahybae, Didelphis aurita, Euryoryzomys russatus, O. nigripes, Oxymycterus judex, S. angouya, Thaptomys nigrita*) were sampled in the Atlantic forest Reserve; and 34 animals of 4 species (*Akodon azarae, Cavia aperea, O. nigripes, Cryptonanus chacoensis*)were sampled in the Pampa Reserve. Sera from these animals were tested by immunofluorescence assay with *R. parkeri* antigen. Overall, 30% (12/40), 18.3% (30/164), and 3% (1/34) animals were seropositive in Cerro Largo, Atlantic forest, and Pampa, respectively.

Conclusions

Results indicate that a spotted fever group *Rickettsia* circulates between small mammals in Cerro Largo (spotted fever-endemic area), and in a lesser extent, in natural Reserves of two biomes of Rio Grande do Sul.

Financial support: FAPESP, CNPq and CAPES.

0226

Molecular detection of *Rickettsia africae* and *Rickettsia felis* from ticks and fleas collected from domestic dogs in Mnisi, South Africa

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Objectives

Rickettsial infections are caused by a variety of obligate intracellular bacteria. *Rickettsia africae,* transmitted by *Amblyomma hebraeum* and *Amblyomma variegatum* ticks, causes African tick-bite fever while *Rickettsia felis* causes flea-borne rickettsioses. Humans are at most risk to both diseases when exposed to infected tick and flea bites during travel to disease endemic areas. DNA from ticks and fleas (103 ticks, 11 pools; 43 fleas, 2 pools) collected from domestic dogs within the Mnisi community, Mpumalanga Province in South Africa were screened for infections with rickettsial pathogens.

Method

Tick species included *Haemaphysalis elliptica*, *Amblyomma hebraeum*, *Rhipicephalus sanguineus*, *Rhipicephalus simus, Haemaphysalis leachi*, and an unspeciated *Ixodes* spp, while fleas included *Ctenocephalides felis strongylus* and *Echidnophaga gallinacea*. DNA analysis detected rickettsial infections in 7 (63.6%) of ticks (*H. elliptica*, *A. hebraeum*, *R. sanguineus*, *R. simus* and *Ixodes* pools) and 2 (100%) of flea pools tested using a genus-specific quantitative real time (qPCR) assay based on the 17-kD antigen gene. Three pools of ticks (27.2%) tested positively on a *R. africae* qPCR assay, while both pools of fleas (100%) were positive on the *R. felis* qPCR assay. Genus-positive PCR products which tested negative with species-specific assays have been sequenced to determine the identity of species involved.

Conclusions

In conclusion, the detection of these zoonotic species, within the vectors of domestic dogs from households, heightens the potential for human exposure to rickettsiae within this rural community in South Africa.

NOTES:

Poster Session II

Tuesday 26th August (17h00-19h00)

0005

***Rhipicephalus rossicus*, a neglected tick at the margin of Europe**

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Objectives

Rhipicephalus rossicus is a three host tick, with a relatively broad host spectrum and typically inhabiting the Eurasian steppe. Although *R. rossicus* has been occasionally reported from people, little attention has been given to its medical importance and vectorial role. Among domestic animals, dogs are probably the most important hosts for ticks and tick-borne pathogens of human importance due to their lifestyle and close contact with people. So far, *R. rossicus* was reported only incidentally as a dog ectoparasite in Europe.

Method

A total of 1120 ticks were collected from household rural dogs between 2012 and 2013 in South-eastern Romania (5 localities in Tulcea and Constanța Counties). Dogs are commonly kept in households, with most individuals roaming freely. Ticks were identified using morphological keys followed by molecular confirmation based on 12S rDNA sequences. Surprisingly, the dominant tick species on dogs was *R. rossicus* accounting for 87.14% of all ticks (32.3-95.3% in different locations). *Rhipicephalus rossicus* had the highest percent of occurrence in dogs in all but one locality. This species had also the highest overall prevalence (mean 0.85) and intensity (9.60, 1-60, SD=13.13772, n=96).

Conclusions

The dominant presence of *R. rossicus* in dogs and its possible occurrence in humans make this tick species an important candidate for further studies to evaluate its host spectrum and vectorial role.

0011

Sympatric occurrence of *Ixodes ricinus*, *Dermacentor reticulatus* and *Haemaphysalis concinna* ticks and their pathogens *Rickettsia* and *Babesia* species in Slovakia

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Objectives

Vojkanad Dunajom in the south-west of the Slovak Republic is a locality with sympatric occurrence of three species of ticks. This study investigated the spatial distribution of *Dermacentor reticulatus*, *Ixodes ricinus* and *Haemaphysalis concinna* ticks in this area, as well as determined the prevalence of *Babesia* and *Rickettsia* species in these tick species considered as potential risk for humans and animals.

Method

Ticks were collected by blanket-dragging over the vegetation from September 2011 to October 2012. Total genomic DNA from ticks was extracted using alkaline hydrolysis. PCR detection of *Rickettsia* spp. was performed amplifying partial regions of the gltA and sca4 genes. *Rickettsia*-positive samples were screened for the presence of *R. helvetica* and *R. slovaca* using qPCR assay targeting fragment of 23S rRNA gene and PCR-RFLP of sca4 gene, respectively. Detection of *Babesia* spp. was performed amplifying fragment of the 18S rRNA gene. Randomly selected amplicons of gltA, sca4 and 18S rRNA genes were purified and sequenced. *D. reticulatus* was the dominant species (67.7%, n=600), followed by I. ricinus (31.8%, n=282) and *H. concinna* (0.5%, n=4) ticks. Rickettsial infection was determined in 10.8% (n=65) and 11.7% (n=33) of *D. reticulatus* and *I. ricinus* ticks, respectively. *Babesia* spp. infection was confirmed in 1.8% (n=11) of *D. reticulatus* and 0.4% (n=1) of *I. ricinus* ticks.

Conclusions

DNA of 6 different pathogenic tick-borne species, *R. helvetica*, *Rickettsia monacensis*, *R. slovaca*, *Rickettsia raoultii*, *Babesia canis* and *Babesia venatorum* were identified in this locality with sympatric occurrence of *I. ricinus*, *D. reticulatus* and *H. concinna* ticks.

0016

Tick and flea-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil

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Objectives

Recently, tick and flea-borne pathogens has been detected in wild carnivores maintained in captivity in Brazilian zoos. Since free-roaming cats are frequently found in Brazilian zoos, they could act as reservoirs for arthropod-borne pathogens, which could be transmitted to endangered wild carnivores maintained in captivity in these institutions. On the other hand, stray cats in zoos may play a role as sentinels to pathogens that circulate among wild animals in captivity.

Method

The present work aimed to detect the presence of Anaplasmataceae agents, hemoplasmas, *Bartonella* species, piroplasmas and *Hepatozoon* sp. DNA in blood samples of 37 free-roaming cats in a Brazilian zoo. Three (8.1%) cats were positive for *Anaplasma* spp. closed related to *A. phagocytophilum*; 11 (29.7%) cats were positive for hemoplasmas (two [5.4%] for *M. haemofelis*, five [13.5%] for '*Candidatus* Mycoplasma haemominutum' and five [13.5%] for '*Candidatus* Mycoplasma turicensis'); 11 (29.7%) were positive for *Bartonella* spp., six (16.2%) were positive *Babesia vogeli* and one (2.7%) for *Theileria* spp. Co-infection with multiple arthropod-borne agentes was observed in sampled cats. None of sampled cats were positive for *Ehrlichia* spp., *Cytauxzoon* spp. or *Hepatozoon* spp. in PCR. This is the first molecular detection of *Babesia vogeli* and *Theileria* sp. in domestic cats in Brazil.

Conclusions

The control of the population of free-roaming cats in these conservation institutions is much needed aiming to prevent the potential transmission to endangered wild animals maintained in captivity, such as wild neotropical wild felids, as well as to human beings visiting zoos.

0017

Three clusters of *Anaplasma phagocytophilum* isolates from clinical cases of French domestic animals revealed by multi-locus sequence analysis

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Objectives

Advance in molecular epidemiology is a great tool to puzzle out complex epidemiological cycles of multi-host pathogens. *Anaplasma phagocytophilum* is a tick-borne bacterium affecting a large range of wild and domestic animals. Here, we aim at characterizing the genetic diversity of *A. phagocytophilum* circulating in French livestock and companion animals to identify if the different hosts carried the same variants and the potentiality of roe deer as reservoir.

Method

We compared the *A. phagocytophilum* strains diagnosed in 120 domestic animals (104 cattle, 13 horses and 3 dogs) with 40 roe deer isolates by multi-loci sequences analysis on 9 loci (ankA, msp4, groESL, typA, pled, gyrA, recG, polA and an intergenic region). The phylogenic analysis allowed the identification of 3 clusters of variants in domestic animals. The two principal clusters included 98% of domestic isolates and were very distant from the roe deer: one of these clusters comprised only cattle and the second grouped cattle, horses and dogs. Only 3 cattle isolates grouped with the roe deer isolates in the third cluster. Geographical factors did not explain this structure.

Conclusions

These results suggest that roe deer do not contribute to the *A. phagocytophilum* spread in clinical cases of domestic animals in France. Further studies should explore if the identified clusters are associated with the severity of the disease observed in domestic hosts. At last, the presence of two clusters in cattle could be explained by the coexistence of two distinct cycles that could have different reservoir hosts.

0018

Molecular detection of *Anaplasma* species in dogs in Colombia

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Objectives

Anaplasma platys and *A. phagocytophilum* are tick-borne pathogens which parasitize platelets and neutrophils, respectively, of both humans and animals. While the former is the etiological agent of Canine Cyclic Trombocytopenia, the later is responsible for Canine Anaplasmosis.

Method

The present work aimed to detect and characterize using molecular techniques the presence of *Anaplasma* species in dogs' blood samples in Colombia. Between December, 2008 and April, 2009, EDTA-blood samples were collected from cephalic vein of 91 dogs from central-western region of Colombia (cities of Bogotá, Villavicencio and Bucaramanga). Blood samples were used in 16S rRNA-*Anaplasma* spp. nPCR and blood smears examinations. One (1%) out of 91 sampled dogs showed inclusion suggestive of Anaplasmataceae agents in the cytoplasm of platelets. Based on PCR followed by sequencing and phylogenetic analysis, A. platys and A. phagocytophilum were detected in two and one dog, respectively.

Conclusions

Although in a low prevalence, *Anaplasma* spp. circulate among dogs in Colombia. The investigation of the zoonotic potential of this strain of *A. phagocytophilum* detected in the present study and the real role of dogs in the epidemiology of human anaplasmosis in Colombia is much needed. This is the first molecular detection of *Anaplasma* spp. in dogs in Colombia.

0021

Epidemiology and evolution of genetic variability of *Anaplasma marginale* in South Africa

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Objectives

South Africa is generally considered as an endemic country for anaplasmosis. Nevertheless, this classification relies on the distribution of the *Anaplasma marginale* tick vectors and serological studies that have shown seroprevalence of anaplasmosis in Limpopo, Free State and North West provinces. However, molecular evidence of anaplasmosis is missing for all the country, except for Free State province. In order to establish effective control measures, it is important to perform epidemiological surveys in order to determine the prevalence and distribution of *A. marginale* in a geographic region.

Method

We analysed by species-specific PCR a total of 250 blood samples from cattle in all the South African provinces except for Free State province. The prevalence of *A. marginale* ranged from 65% to 100%, except in Northern Cape Province with negative results. Correlation was found between the genetic diversity of *A. marginale* MSP1a and its prevalence. MSP1a genetic diversity showed to evolve under negative and positive selection and the 23 new tandem repeats found in South Africa evolved from the extant tandem repeat 4.

Conclusions

One of the proposed strategies to control anaplasmosis is the development of *A. marginale* major surface protein 1a (MSP1a) based vaccines which recently have recovered new attention. Despite the MSP1a genetic variability, some types of tandem repeats were found conserved among the *A. marginale* strains and also low variable peptides in MSP1a tandem repeats were identified. Our results confirm by molecular ways the endemicity of anaplasmosis in South Africa and will contribute to the rational design of MSP1a based vaccines.

0028

Dynamics of individual exposure to *Coxiella burnetii* infection in a Q fever endemic red deer (*Cervus elaphus*) farm

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Objectives

Infection dynamics of *Coxiella burnetii* in Q fever endemic herds in the long-time scale may be influenced by a trade-off balance between infection pressure and herd immunity, among other factors. Variations in the rate of susceptible-to-immune individuals in the herd with time may drive shedding patterns, and therefore infection pressure and transmission rates. Analysing factors driving *C. burnetii* dynamics in endemic herds will help understanding host-*C. burnetii* interaction traits to improve Q fever control schemes.

Method

From 2008 to 2013, 217 red deer from a Q fever endemic farm were followed at least twice a year since 7 up to 62 months of life for quantification of *C. burnetii* antibodies (ELISACOXLS, ThermoFisher Scientific, USA). Average sample-to-positive control ratio (a proxy for antibody levels) increased with animal age - from 16.9±24.0 for 7 month-old to 108.1±37.1 for 62 month-old individuals. Increasing seroprevalence from winter to summer constituted the general annual pattern. Most individuals (95.8%) alternated seropositive with seronegative periods during the study; very few (4.2%) remained seropositive or seronegative in at least eight consecutive samplings from 7 months of age.

Conclusions

The humoral immune response to *C. burnetii* infection in red deer is affected by age, which may be related both to increasing immune capacity and to cumulative effects. The observed annual seroprevalence pattern suggests that *C. burnetii* in deer is shed - and transmitted - mainly around the calving season (late spring). The short average duration of *C. burnetii* antibodies suggests that early vaccination approaches to control Q fever in red deer should be applied during winter months, when antibody levels are expected to lower.

0034

Fourier-transformed remote sensing data has superior performance over other abiotic variables to describe the niche of ticks.

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Objectives

The interest in modelling the environmental niche of pathogens-transmitting ticks increased over the past years. However, data quality and methodological concerns related to using climate data to capture such niche have not been addressed adequately. The use on inadequate sets of explanatory variables may inflate the model and produce unreliable results. This study evaluated different sets of covariates to capture the environmental relationships for the ticks *Ixodes ricinus* and *Hyalomma marginatum*.

Method

We compared the potential of MODIS remotely sensed and interpolated gridded covariates. We used time series of data (monthly values of variables), a Principal Components Analysis (PCA) and a harmonic regression to process covariates. We assessed model inflation resulting from spatial autocorrelation (SA) and collinearity (CO) of the covariates. Reliability was measured via AUC, autocorrelation by Moran's *I* and collinearity by the variance inflation factor (VIF).

Conclusions

Monthly series of interpolated climate always captured better the niche of ticks. However, the SA was 2 times higher and the maximum VIF was 30 times higher in interpolated than in MODIS-derived covariates. Monthly series had higher SA and CO than their transformations by PCA or Fourier. Interpolated datasets had always higher SA and CO than remotely sensed covariates. Satellite-derived information has fewer drawbacks to model the environmental niche of ticks. A harmonic regression of remotely-sensed data is the most promising tool, because it retains both biological and statistical meaning.

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0043

A survey of severe fever with thrombocytopenia syndrome (SFTS) virus detection from wild animals and Ixodidae ticks in Korea

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Objectives

Severe fever with thrombocytopenia syndrome (SFTS) is caused by SFTS virus (SFTSV) a novel bunyavirus reported to be endemic in central and north-eastern China, southern Japan and Korea. To investigate the infections of SFTSV in wild animals and ticks, we sampled serum and ticks from wild animals in Korea, from May to October 2013.

Method

SFTSV-specific genes were amplified and sequenced from animal sera and ticks by One-step RT-nested PCR method. Collected serum samples from 6 species wild animals (5 mammals and 1 bird species) were total 91 animals. Mammals were 21 Korean water deer (*Hydropotes inermis*), 3 Siberian roe deer (*Capreolus pygargus*), 5 goral (*Naemorhedus caudatus*), 7 raccoon dog (*Nyctereutes procyonoides*) and 54 wild boars (*Sus crofa*). Bird was 1 Carrion crow (*Corvus corone*). Infection rate of SFTS virus in wild animals was 3.30% (3 of 91 animals). Korean water deer was 4.76% (1 of 21, collection date was August 5, 2013). Wild boars were 3.70% (2 of 54, collection dates were August 5 and 8, 2013). Ticks were collected total 891 from 65 wild animals, 9 species {6 species of mammals (Korean water deer, Siberian roe deer, raccoon dog, badger, goral, hedgehog) and 3 species of birds (crow, Eurasian eagle-owl, sparrowhawk)}. Total 667 *Haemaphysalis longicornis* were collected 120 larvae, 174 nymphs, 63 adult males, and 310 adult females. Total 180 *Haemaphysalis flava* were collected 23 larvae, 31 nymphs, 68 adult males and 58 adult females.

Conclusions

Total 44 *Ixodes nipponensis* were collected 3 larvae, 2 nymphs, 13 adult males, 26 adult females. Minimum field infection rate (MFIR) of SFTSV in tick species is 4.98% (most of positive tick samples were collected from May and June, 2013). MFIRs of SFTSV were 4.51%, 2.22% and 22.73% from *H. longicornis, H. flava* and *I. nipponensis*, respectively. Average MFIRs of SFTSV in Korea was 4.98%. Genotypes of SFTSV were identified 2 or more presence from wild animals and ticks. This report describes the first detection of wild animals with SFTS virus in Korea.

0046

A survey of ticks (Acari: Ixodidae) and severe fever with thrombocytopenia syndrome (SFTS) virus infection in National Parks

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Objectives

Severe fever with thrombocytopenia syndrome (SFTS) is caused by SFTS virus (SFTSV) which is in the Phlebovirus genus in the Bunyaviridae family, and reported in central and northeast of China, southern Japan, and Korea. This paper is first to report the discovery of the SFTS virus from the ticks collected from the trail and forest of Korean national parks from July to October 2013.

Method

SFTSV-specific genes were amplified using One-step RT-nested PCR method. Total 1,204 ticks, 287 *Haemaphysalis longicornis*, 151 *H. flava*, 755 *H.aemaphysalis* larvae, 10 *Ixodes niponensis*, and 1 *Ambylomma testudinarium,* which were collected from 81 collections from 4 national parks, and were analyzed. Three pools were found positive for SFTS virus (2 pools from Odaesan and 1 pool form Mudeungsan). SFTS virus positive ticks were found in 2 species, *H. longicornis* nymphs, and *H. flava* adults and nymphs, were in the developmental stages of nymph and adult. Except for the larvae, the minimum field infection rate (MFIR) was 10% for Odaesan, 0.47% for Mudeungsan, and 0% for Gyeryongsan and Naejangsan averaging 0.67%. From the SFTS virus positive ticks sampled from the National Parks, the nucleocapsid protein S fragment sequences appeared to be 2 genotypes that were observed, the genotypes that appeared in OPark44 (Odaesan) and MPark8 (Mudeungsan) had 99.7% matching DNA with the SFTS virus isolated from Korean human (one of 346 bases was different position), the genotype that appeared in OPark43 (Odaesan) showed 93.6% matching (when compared to the virus isolated from Korean human, the 22 of 346 bases were different) and appeared to be a different genotype.

Conclusions

When translated into amino acid sequences, the genotype MPark8 (Mudeungsan), matched with the Korean patients and the occurrences of China. One and two amino acid bases differed for OPark44 (Odaesan) and OPark43. Of the 2 nucleocapsid protein S segment genotype of the SFTS virus from collected ticks in Korea National Parks, OPark44 (Odaesan) and MPark8 (Mudeungsan) genotype matched with one strain from Chinese isolate but OPark (Odaesan) appeared to be different from the strain reported from China and Japan (Further research is required).

0047

Detection of severe fever with thrombocytopenia syndrome (SFTS) virus from domestic pigs in Korea

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Objectives

Severe fever with thrombocytopenia syndrome (SFTS) is caused by SFTS virus (SFTSV) a novel bunyavirus reported to be endemic in central and north-eastern China, southern Japan and Korea.

Method

To investigate the infections of SFTSV in domestic pigs, we sampled pig serum from 8 provinces in Korea, from July to October 2013. Selection of the regions for blood samples from domestic pigs were mountain regions, which were collected from 240 pigs (82 domestic pigs, 138 black pigs and 20 domestic wild boars) from conventional pig farms from 8 provinces of Korea excluding Jeju-do (island). We collected 5 blood samples from each pig farm from 6 farms per province and total 30 blood samples from each province. SFTSV-specific genes were amplified and sequenced from domestic pig sera by One-step RT-nested PCR method. The amplified genes were sequenced. Infection rate of SFTS virus in domestic pigs was total 1.67% (4 of 240 domestic pigs). The infection rates of SFTS virus in each province were 3.33% (1 of 30 domestic pig), 6.67% (2 of 30 domestic pigs) and 3.33% (1 of 30 domestic pigs) in Gyeonggi-do, Gyeongsangbuk-do and Gyeongsangnam-do provinces, respectively. SFTS virus was identified 2 genotypes in nucleotide and translated amino acid sequences from domestic pigs by sequence comparison and phylogenetic trees.

Conclusions

This is first to report the discovery of the SFTS virus from the domestic pig in Korea.

0048

Survey on distribution of ticks in domestic pigs, wild boars and their habitats

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Objectives

Over the past several years, there was a substantial increase in the number of cases of known and novel tick-borne infections in animals and humans in the Republic of Korea (ROK). In this survey conducted in July to October 2013, distribution of ticks in domestic pig and wild boar in Korea were determined. Mountain terrain form eight different provinces in the ROK excluding Jeju-do were the selected regions for collecting ticks from domestic pigs and wild boars.

Method

Then ticks and blood sample was collected from small pig farms or conventional pig farms located around the mountain terrain. Flagging and sweeping methods were used in areas of wild boar appearances for collection. A total of 9,606 ticks were collected from habitats and conventional pig farms. 9,028 ticks belonging to 2 genera and 4 species (4,347 *Haemaphysalis longicornis,* 4,172 *H. flava,* 508 *Ixodes nipponensis,* and 1 *I. turdus*) were collected at the habitats of wild boar which were 8 selected sites of 8 provinces. Of the 578 ticks (538 *H. longicornis,* 40 *H. flava*) were collected in pastures and forests near the conventional pig farms. Hard and soft ticks were not found in domestic pigs. A total of 231 hard ticks (79 *H. longicornis*, 107 *H. flava,* and 45 *Amblyomma testudinarium)* were collected from 72 wild boars (Korean wild boar, *Sus scrofa*). Then blood samples were collected from 72 wild boars from 8 provinces of Korea. Also, blood samples were collected from 240 pigs (82 domestic pigs, 138 black pigs, and 20 domestic wild boars) from conventional pig farms from 8 provinces of Korea.

Conclusions

In this study, we could not find any soft ticks from both domestic pigs and wild boars, also, their habitats by sweeping and flagging methods in the ROK.

0052

Effect of climatic factors on the occurrence and dominance of ticks in the karst region of Slovakia

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Objectives

Ticks represent the most important vector - borne group in Central Europe. There are three epidemiologically important genera of ticks in Slovakia: *Ixodes*, *Dermacento*r and *Haemaphysalis*. The temperature and relative humidity significantly influence the occurrence and abundance of seeking ticks. The aim of our study was to analyse the influence of climatic factors on the activity and abundance of ticks in Karst region of southern Slovakia.

Method

Ticks were collected by flagging methods in two-week intervals. Research was conducted between February 2011 and February 2012 near village Hrhov. We obtained 802 ticks belonging to six species with predominance three of them: *Ixodes ricinus* (59.4 %), *I. frontalis*, *Dermacentor marginatus* (12.7 %), *D. reticulatus*, *Haemaphysalis inermis* (23.9 %) and *H. concinna*. Sporadically collected species - *I. frontalis*, *D. reticulatus* and *H. concinna* were excluded from our analyses. Our results reflect the important changes in species diversity and abundance during different seasons. It shows the different ranges and optimum values of climatic factors on activity of individual tick species.

Conclusions

From the correlation analyses between the tick occurrence and values of climatic factors, we found that temperature and saturation deficiency significantly correlate with abundance of two tick species - *I. ricinus* and *H. inermis*.

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0055

Cattle and ticks in the Brazilian wildlife rich Pantanal: impact on environmental infestation

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Objectives

Pantanal is a large floodplain with an estimated area of 138.183 km² in Brazil. It has a mosaic of phytophysiognomies from the Cerrado biome, the Brazilian savannah. It is overwhelmingly privately owned and extensive cattle ranching is the main economic activity. At the same time, it has an extraordinarily rich wildlife. Cattle (*Bos indicus*) grazing many times occur on native pasture and bovines rest in native forestall formations. Therefore, cattle are in permanent contact with wildlife.

Method

In this work, we evaluated cattle tick infestation and environmental infestation at both cattle raising areas and a natural reserve. Furthermore, suitability of cattle to *Amblyomma sculptum* nymphs (from *Amblyomma cajennense* complex) the most prevalent tick species in the wild and found on bovines as well, was experimentally assessed. Three tick species were found in the environment, overwhelmingly *Amblyomma sculptum* followed by *Amblyomma parvum* and *Amblyomma ovale*. Nymphs and adults ticks were found in the dry season but only adults in the wet. In the wet season forested formations with cattle were significantly more infested by adult ticks then those from the reserve. From 75 bovines 2,100 ticks from three species were collected; *Rhipicephalus microplus* (65.1%), *Amblyomma sculptum* (31.2%) and *Amblyomma parvum* (3.6%). *Morisita-Horn* index, calculated for *Amblyomma*, indicated similarity between tick fauna from environment and on cattle during both seasons. *A. sculptum* nymphs attached readily and engorged on calves during experimental infestations.

Conclusions

In conclusion, cattle in Pantanal are infested by tick species from wild environment and from these effectively feed *A. sculptum* nymphs.

0065

The first isolation of *Brucella melitensis* (Rev. 1) vaccine strains from adult small ruminants in Sicily

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Objectives

Brucellosis in sheep and goats, caused by *B. melitensis*, is a serious zoonosis of socio-economic importance worldwide. The disease causes abortion in small ruminants and it can be spread to humans who handle material from infected animals or by consumption of raw milk and milk products. Vaccination continues to be the recognised method to control the spread of disease, reducing abortions and risks of human infection. With this in mind, when examinations were carried out in order to confirm the presence of disease, the opportunity was taken to fully identify the strains circulating in the Sicilian herds and flocks.

Method

Out of a total of 51 *Brucella* strains confirmed in IZS in 2010 from sheep and goats, 5 samples (all isolated from adult animals)were classified, biochemically and according to OIE procedures, as *B. melitensis*Rev.1 strains. Strains were isolated from material coming from three different Sicilian districts: Messina, Caltanissetta and Catania. In total, *B. melitensis* Rev.1 was isolated in five different outbreaks, from ten females**.** All 10 animals were serologically positive for brucellosis and came from farms that were previously notified as brucellosis infected. Based on the criteria for the determination of *Brucella* species and vaccine status using SNP based Taqman real time PCR assays described by Gopaul *et al.,* 2008 and Gopaul *et al.,* 2010, all isolates were unequivocally identified as *B. melitensis* Rev1. The results from Bruceladder testing based on the protocol described by Mayer Scholl *et al.,* (2010) also indicate that the five isolates tested were *B. melitensis* Rev1 from the distinct banding profile generated.

Conclusions

By identifying *B. melitensis* Rev.1 isolates, this report has complicated the eradication plans for the disease because none of the ten animals from which Rev.1 was isolated were officially vaccinated. The farms where Rev.1 was found did not officially report any relevant clinical signs related to the disease such as abortion. The origin of the Rev.1 vaccine strain from these unvaccinated Sicilian sheep and goats remains to be resolved. It is known that in extensive livestock production typical of Southern regions, sheep from different flocks often graze together during the transhumance period, which can represent a source of spread.

0067

The development and partial validation of an OpTSGP1 ELISA

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Objectives

Argasid ticks (i.e. *Ornithodoros spp.*) are distributed throughout the Southern African Development Community (SADC). The argasid ticks can transmit African swine fever virus (ASFV), one of the most destructive haemorrhagic diseases in domestic pigs. ASFV is transmitted and maintained in an ancient sylvatic cycle involving wild sued species and the tick vectors of the *Ornithodoros* spp. The tick vectors co-habit the burrows used by the warthogs where they feed on the warthogs' blood and transmit the ASFV during these feeding sessions.

Method

These ticks may be transported by their hosts to new areas where they infect new populations of warthog or domestic pigs. Infected ticks are able to re-infect pigs that are reintroduced on farms that previously had an outbreak. Detection of specific antibodies against tick salivary proteins from the animal hosts living in the area under study would provide a more convenient diagnostic tool for evaluation of the interaction between ticks and pigs. The objective of the present study was to develop an ELISA to detect antibodies to the OpTSGP1agrisid salivary protein in pigs following exposure to Ornithodoros ticks.

Conclusions

This study may help to detect the presence or interaction of ticks with pig hosts.

0068

Molecular detection and characterization of *Babesia* species in cervids and in ticks infesting cervids in Lithuania

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Objectives

In Europe, babesiosis has been reported in human and some domestic and free-ranging mammal species. Wild populations of Cervidae species are suspected to be reservoir hosts of some *Babesia* pathogens. The aim of our study was to investigate the presence of *Babesia* species in native red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and introduced sika deer (*Cervus nippon*) and fallow deer (*Dama dama*) occurring in farmed and free living populations in Lithuania. Moreover, engorged ticks from cervids and questing ticks from deer farms territories were screened for *Babesia* pathogens.

Method

Blood and spleen samples of a total of 72 animals (1 moose, 3 fallow deer, 4 red deer, 20 sika deer and 44 roe deer) were collected during the period from 2011-2013. A total 207 ticks were collected from animals and vegetation. Different regions of the 18S rRNA gene of the genus *Babesia* were amplified and sequenced. *Babesia* species were detected in 72.7% of roe deer, 5.0% of sika deer, in 3 specimens of red deer, 2 fallow deer and in 19,4% and 29,4% of ticks from cervids and questing ticks, respectively. Sequence analyses indicate the presence of *B. venatorum*, *B. microti* in *Ixodes ricinus* ticks, and *B. venatorum*, *B. capreoli* and *B. divergens* in cervids.

Conclusions

The present study represents the first molecular characterization of potentially zoonotic *B. microti*, *B. venatorum* and *B. divergens* in Lithuania. The high infection rate of babesias in cervids supports their role as reservoir hosts for these pathogens.

0070

Borrelia burgdorferi s.l. among questing ticks and small mammals in northern Spain natural reserve (Sierra del Sueve-Asturias)

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Objectives

Lyme borreliosis is an arthropod-borne disease distributed worldwide. The first reported cases of human Lyme disease in Spain were published in 1977 (Uruñuela-Bernedo and Diaz Sosa, 1977) and 1987 (Uria et al., 1987). Small mammals have been described as important reservoir host for Borrelia burgdorferi sensu lato in different European studies. However, low prevalence of infection (0.5%) was found in a Basque Country study (Gil et al., 2005). This study was conducted to assess the presence of *Borrelia burgdorferi s.l.*, the causative agent of Lyme Borreliosis, in questing ticks and small mammals in Asturias.

Method

Questing ticks were collected from six different areas that covered most of the ecological biotopes in the natural reserve "Sierra del Sueve" located in the province of Asturias. Ticks were collected from vegetation by blanket dragging (1 x 1m). Sampling at each site was conducted twice a month from April 2010 to December 2013. Small mammals were captured, between October 2012 and October 2013, in the same areas previously described for the ticks' blanket dragging. Sherman traps were placed overnight and trapped animals, were brought to the laboratory for classification (19 *Apodemus* sylvaticus, 2 *Microtus lusitanicus*, 1 *A. flavicolis*, , 1 *M. agrestis*, 1 *Microtus sp.*, 1 *Crocidura russula* and1 *C. suaveolens*), tick count (ranging 0-8 *I. ricinus* larvae) and tissue collection (ear, lung, heart, liver, spleen, kidney and urinary bladder) for PCR. 920 nymphs (184 pools) and 60 adults (individual samples) ticks, as well as 26 small mammals' tissue pools, were examined by polymerase chain reaction (PCR) for the presence of *Borrelia burgdorferi* s.l. (Clark et al. in 2005). In positive PCR samples, the rrs-rrlA intergenic spacer was sequenced for species identification (Bunikis et al., 2004).

Conclusions

Borrelia burgdorferi s.l. was detected from 0.8-3.8% nymphs and 3.3% adults collected along four years (2010-2013). *Borrelia burgdorferi s.l.* was also detected from 11.5% (3/26) small mammals. Two different genospecies (*B. afzelli* and *B. garinii*) were identified from the questing ticks. The detection of *Borrelia burgdorferi s.l.* among questing ticks and small rodents, as well as the presence of large populations of wild and domestic animals indicate that the risk of infection in this area is relevant. This is also in accordance with clinical reports of Lyme disease from local hospitals.

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0099

Update on epidemiology of canine piroplasmosis in southern France

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Objectives

Canine piroplasmosis is an emerging disease caused by Babesia and Theileria protozoans, also called piroplasms, transmitted by Ixodid ticks. In northern France, Babesia canis, transmitted by Dermacentor reticulatus, is considered as the main etiological agent of the disease. In southern France, where infection of dogs and Rhipicephalus sanguineus ticks by Babesia vogeli was recently confirmed, no comprehensive study was performed yet.

Method

This work presents results of a survey conducted from 2010 to 2012 in this area, in which 155 dogs were enrolled. From them, 140 bloods and 635 ticks (including 574 R. sanguineus s.l. and 33 D. reticulatus) were retrieved. All blood and D. reticulatus samples as well as 242 R. sanguineus s.l. specimens were screened for the presence of piroplasms by PCR amplification of 18S rDNA. Nineteen dogs (13.6%) were found positive for Babesia vogeli, 18 (12.9%) for Babesia canis and 1 (0.7%) for Theileria annae. Twenty six (10.7%) R. sanguineus harbored B. vogeli and 4 (1.7%) B. canis whereas B. canis was the only piroplasm detected in D. reticulatus (N=3; 9.1%). Strikingly, significant differences in prevalence of B. vogeli infections, in both dogs and R. sanguineus, were found between regions that could not be explained by genetic variability of ticks (12S and 16S mt-rDNA) or pathogens (18S rDNA).

Conclusions

Overall, this study confirmed that B. canis and B. vogeli piroplasms circulate in southern France. Further studies focusing on genetic and microbiota of R. sanguineus ticks should be conducted to explore other biological interactions that may explain differences observed.

0114

Ixodidae distribution in relation with climate and environmental factors in the natural reserve of Monte Pellegrino in Sicily, Italy

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Objectives

The development of tick control measures is related to their epidemiology knowledge. This study concerns a spatial-temporal distribution analysis of free living ticks in the natural reserve of Monte Pellegrino, in Palermo (Italy).

Method

Ticks were collected with dragging methods and identified, during a one-year monitoring from June 2012 to May 2013. Monthly abundance was evaluated in six different sites, with different environmental characteristics. A total of 1.727 ticks were collected comprehending six different species. June 2012 showed the highest number of ticks (324 exemplars). Site n. 5, situated in an artificial forest of pines and cypresses at 280 m above sea level, and presented the highest number of ticks (n. 706). *Ixodes ventalloi* was the most abundant species with 679 exemplars, collected from September 2012 to April 2013 with a peak in January 2013. The site n. 6, presenting a little seasonal lake and an artificial forest of pines and eucalyptus at 400 m above sea level, had the greatest variety of tick species. Sites having similar characteristics showed similar tick distributions. For example, *Hyalomma lusitanicum* was captured almost exclusively in sites n. 5 and n. 6, from June 2012 to November 2012. Tick density was related to environmental and climatic factors (altitude, land cover, vegetation, temperature and precipitation). Monthly maps with proportionate circles were developed using the geographical information systems.

Conclusions

The study constitutes a premise for additional researches including hosts distribution analysis, correlation with pathogens in ticks, map risk processing and correlation with microclimate.

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0115

Ticks and rodents with *Anaplasma phagocytophilum* and *Candidatus* Neoehrlichia mikurensis infection in Southern Hungary

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Objectives

Anaplasma phagocytophilum has been long known as the pathogen causing "tick-borne fever". Wild ruminants and rodents have the biggest role in pathogen life cycle and ticks can infect domestic species and also humans. *Candidatus* Neoehrlichia mikurensis is a new human pathogenic bacterium belonging also to Anaplasmataceae first detected in the late 1990s. The key role of rodents as reservoir host has recently been proven.

Method

In order to investigate these tick-borne bacteria in Southern Hungary we collected small mammals with live-traps (2010-2013) and questing ticks with flagging in 2012. We euthanized the small mammals and collected tissue samples and removed all the ectoparasites and stored in 70% alcohol. We found low tick infestation (8%). Samples were analysed for *A. phagocytophilum* and *Candidatus* N. mikurensis with multiplex quantitative real-time PCR. A part of msp2 gene from *A. phagocytophilum* and the groEL heat shock protein gene from *Candidatus* N. mikurensis were amplified to examine the infection. We found in the tissue samples 7% (skin) and 5.27 % (spleen) *A. phagocytophilum* and 2.25% (skin) and 2.87% (spleen) *Candidatus* N. mikurensis infection. Among questing ticks we found three *Ixodes ricinus* (female, male, nymph) with *Candidatus* N. mikurensis (1.85%) and five ticks with *A. phagocytophilum* (two *Dermacentor reticulatus* females, two *Haemaphysalis concinna* females, one *I ricinus* male) (3.1%) infection. We found one *Ixodes ricinus* nymph removed from male *Apodemus flavicollis* with *A. phagocytophilum* infection.

Conclusions

This study showed the presence of these pathogens with relatively low prevalence in questing ticks, engorged ticks and rodent tissue samples.

0133

Three-years study of *Babesia* sp. and *Anaplasma phagocytophilum* in questing ticks in Southwestern Slovakia.

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Objectives

Babesiosis caused by intraerythrocytic parasites of the genus *Babesia*, and anaplasmosis caused by the obligate intracellular bacterium *Anaplasma phagocytophilum*, are emerging zoonotic diseases of veterinary and medical importance and are transmitted by ixodid ticks. In this study we monitored the occurrence of these pathogens in questing *Ixodes ricinus* and *Haemaphysalis concinna* in two areas of South-western Slovakia that are differently affected by human activities and comprise different reservoir hosts.

Method

Ticks were collected during 2011-2013 by blanket-dragging at two sites in the Small Carpathians Mts: the recreational area of the Bratislava forest park, and non-fragmented woodland. The distance between the two sites is cca. 40 km. By using PCR-based methods (target gene 18S rRNA) *Babesia* sp. was detected in 43 (1.5%) out of 2823 ticks from the forest park and in 47 (2.1%) out of 2251 ticks from the woodland site. By sequencing *Babesia*-positive samples *B. microti, B. venatorum, B. crassa, B. canis, B. capreoli* and *Hepatozoon canis* were identified. For detection of *Anaplasma phagocytophilum*, Real-Time PCR targeting the msp2 gene was used. In Bratislava forest park 346 (12.2%) out of 2825 ticks were positive for *A. phagocytophilum*, in the woodland site 69 (3.1%) out of 2251 ticks were *Anaplasma* positive.

Conclusions

The study confirms spatial and temporal differences in the prevalence of the studied pathogens. Knowledge about the prevalence of these infectious agents in ticks is an important prerequisite for risk assessment of diseases.

Acknowledgments

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0135

Molecular detection of tick-borne pathogens in rodents and rodent-attached ticks in SW Slovakia.

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Objectives

In Europe, rodents (especially of the genera *Apodemus* and *Clethrionomys*) are important reservoirs of pathogens such as *Borrelia burgdorferi* s.l., *B. microti* and may also act as reservoirs of *Anaplasma phagocytophilum*. Borreliosis, babesiosis and granulocytic anaplasmosis are zoonotic diseases with a natural enzootic cycle involving ticks and vertebrate hosts. In this study the presence of different tick-borne microorganisms was monitored in rodents in South-western Slovakia.

Method

Rodents were trapped with live traps at two sites in the Small Carpathian Mts. A total of 407 and 8 rodents belonging to five species were trapped in the research area in 2012 and 2013, respectively. *Apodemus flavicollis* and *Clethrionomys glareolus* prevailed. In 2012, rodents were infested with ixodid ticks (n=764) - *Ixodes ricinus* (94.1%) and *Haemaphysalis concinna* (5.9%), in 2013 (n=207) - *I. ricinus* (99.5%) and *H. concinna* (0.5%). Spleen samples from rodents were examined by PCR for presence of *Babesia, Borrelia*, and *A. phagocytophilum*. In total, 8.8% were infected with *Babesia* sp. (dominance of *B. microti* and *Hepatozoon* sp.), 0.3% with *A. phagocytophilum* and 0% with *B. burgdorferi* s.l. Host-attached ticks were infected with *Babesia* sp. (3.3%; dominance of *B. microti*), *A. phagocytophilum* (0.5%) and *B. burgdorferi* s.l. (4.1%; *Borrelia garinii* and *B. afzelii*).

Conclusions

Our results show the involvement of rodents in the natural endemic cycles of tick-borne pathogens in SW Slovakia. Further screening of rodent tissues and molecular identification of strains of pathogenic microorganisms is in progress.

Acknowledgments

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0136

Clinical and laboratorial findings in an outbreak of tick-borne disease in naïve Assaf dairy sheep in northern Spain

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Objectives

This study was designed to investigate an outbreak of disease occurred in Assaf sheep introduced into a Latxa sheep flock in the Basque Country. Latxa, the native dairy sheep breed of the Basque Country, is semi-extensively managed in mountain pastures in contact with ticks, whereas Assaf sheep are reared in intensive management systems rarely exposed to ticks. In December 2012, 120 Assaf sheep were purchased. Soon after animals started grazing (July), ticks were observed and clinical signs appeared. Main symptoms consisted of hyperthermia, anorexia, lethargy, tremors, weakness and haemoglobinuria, and animals died in 48h.

Method

Only Assaf sheep were affected but not Latxa, and in August more than 50% of the animals had died. Samples from 13 ewes (2 dead) and 10 lambs were collected and subjected to a panel of general laboratory methods and specific methods for common tick-borne pathogens of livestock that included a multiplex real-time PCR for *Anaplasma* species, and a suspension microarray for ovine piroplasms. *Babesia ovis* (12 animals), *Theileria ovis* (6) and *Anaplasma ovis* (4) were identified, both as single as mixed infections. Anaplasmas and/or piroplasms were detected in 7/10 lambs and 12/13 ewes. Most of the animals infected with *B. ovis* had a marked decrease in the values of the red blood cell parameters. Microscope examination of blood smears revealed piroplasms compatible with *B. ovis*. Ticks collected from the animals were identified as *Riphicephalus bursa*, vector of *B. ovis*. Other haemolytic pathologies (clostridial disease, copper poisoning and leptospirosis) were ruled out, and babesiosis by *B. ovis* was diagnosed.

Conclusions

Special attention should be paid when introducing naïve animals into a piroplasmosis endemic area.

This study was supported by INIA RTA2011-00008-C02-02 and the European Regional Development Fund (ERDF).

0137

The prevalence and the seasonal dynamics of tick-borne pathogens in questing ticks from the Slovak Karst region, Central Europe.

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Objectives

The aim of this study was to investigate the prevalence of infection and the seasonal dynamics of *Borrelia* spp., *Babesia* spp. and *Anaplasma phagocytophilum* in questing ticks (*Ixodes* spp., *Dermacentor* spp., *Haemaphysalis* spp.) collected in the Slovak Karst National Park (48°34.899 N, 20° 46.743 E) in eastern Slovakia. This area is characterized by the presence of several endemic plant and animal species, and also by the co-occurrence of at least 5 tick species.

Method

Ticks were collected in two-week intervals, during the one year period (2011 - 2012). In total, 660 ticks of four species were examined for the presence of tick-borne pathogens by the molecular methods. *Ixodes ricinus* ticks carried the widest spectrum of pathogens with the highest prevalence of spirochetes from *Borrelia burgdorferi* s.l. complex (±12.0%). The predominant genospecies were *B. garinii* (±40.0%) and *B. afzelii* (±37.0%), followed by *B. lusitaniae*, *B. valaisiana* and *B. burgdorferi* sensu stricto. In addition, one *Dermacentor marginatus* tick tested positive for *B. afzelii* and one *Haemaphysalis inermis* carried *B. valaisiana*. The second, most prevailing pathogen of *I. ricinus* ticks was *Babesia* spp. (±8.0%). Sequencing allowed distinguishing between *B. microti* "Jena strain" (±65.0%) and *B. venatorum* (22.0%). *A. phagocytophilum* was confirmed in almost ±3.0% of tested *I. ricinus* ticks.

Conclusions

This study confirmed that the studied area represents a significant tick-borne diseases hotspot. For more ecological details and data about the seasonal dynamics of ticks, see the poster of Stanko M. et al.: "Effect of climatic factors on the occurrence and dominance of ticks in the karst regions of Slovakia.

The research was supported by the Slovak Research and Development Agency APVV 0267-10, VEGA 2/0113/12, VEGA 1/0390/12 and VEGA 2/0055/11.

0140

Abundance of *Ixodes ricinus* in an urban and woodland area in Southwestern Slovakia

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Objectives

In recent years, tick-borne diseases have (re)emerged and spread in Europe due to global climatic and socio-economic changes. *Ixodes ricinus* is the principal vector of numerous microbial pathogens of medical and veterinary importance. Shift in the distribution of *I. ricinus* northward and to higher altitudes was observed and ticks became more abundant in city and forest parks, leading to increasing risk of exposure of humans and domestic animals to infected ticks.

Method

Six 100 m transects were selected across an urban-natural gradient in SW Slovakia (Bratislava - Small Cartpathian Mts). Ticks were dragged along transects with a 1 m^2 blanket during the season of highest activity (April-June) and in September-October 2011-2013. A total of 6,015 *I. ricinus* were collected (3,435 and 2,580 in the urban-suburban and natural habitat, respectively). In addition to *I. ricinus*, *Haemaphysalis concinna* was present in the area and comprised up to 3.5% of the tick collections. Spatial and temporal variation in *I. ricinus* abundance and differences in the subadult/adult ratio were observed between the habitats, but also between transects within the same type of habitat. In 2011 and 2012, highest tick abundances were registered in April-May, whereas in 2013 in June. The numbers of ticks per transect/collection date varied between 2 and 340 ind. in the urban-suburban habitat and between 0 and 300 ind. in the natural habitat.

Conclusions

The observed differences indicate that local microclimate and host spectrum affect the abundance of *I. ricinus* populations.

Acknowledgements: This work was supported by FP7 project EDENext (No. 261504) and grant APVV DO7RP–0014–11.

0142

Prevalence of *Candidatus* Neoehrlichia mikurensis in questing ticks and rodents in Southwestern Slovakia

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Objectives

Candidatus Neoehrlichia mikurensis (Rickettsiales, Anaplasmataceae) is considered as an emerging tick-borne pathogen and has caused severe disease in immunocompromised humans in Central and Northern Europe. *Ca.* Neoehrlichia mikurensis has been detected in *Ixodes ricinus*, but knowledge on its distribution and reservoirs in Europe is still limited. Rodents seem to play an important role as reservoirs of the bacterium.

Method

In this study, questing *I. ricinus* ticks were collected and rodents were live-trapped at six sites of the Small Carpathian Mts, on a suburban-natural gradient between 2011 and 2013. Tick and rodent spleen DNA were screened by real-time PCR amplifying the *groEL* gene of *Ca.* Neoehrlichia mikurensis. The bacterium was detected in questing *I. ricinus* nymphs and adults. Depending on the site and year, the overall prevalence of *Ca.* Neoehrlichia mikurensis in ticks ranged from 0% to 4.48%, with higher average prevalence in the woodland area than in the recreational area of the Bratislava forest park. The presence of the bacterium was confirmed in spleens of 4 out of 5 captured rodent species - *Apodemus flavicollis, Clethrionomys glareolus, Microtus arvalis, and Mircrotus subterraneus*, except *A. sylvaticus*. Prevalence in spleens of all rodent species captured in the woodland area was higher than in rodents from the forest park, with an overall prevalence of 10.76% in the woodland and 3.66% in the forest park.

Conclusions

The study confirmed circulation of *Ca.* Neoehrlichia mikurensis in SW Slovakia and a potential risk of human infections.

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0161

Transplacental transmission of *Babesia caballi* in Thoroughbred foals in Trinidad

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Objectives

Equine piroplasmosis caused by *Theileria equi* and *Babesia caballi* is endemic in Trinidad, West Indies. Transmission occurs mainly by ticks of the genus *Ixodidae*. *T. equi* can also be transmitted transplacentally, however transplacental transmission of *Babesia caballi* is unknown. In countries where equine piroplasmosis is endemic transplacental transmission may be a more common occurrence than previously suspected. This study aims to investigate transplacental transmission of *Babesia caballi* from thoroughbred mares naturally infected via the tick vector.

Method

Whole blood and serum samples were collected from 115 mares in the fifth month of pregnancy. Samples were also collected from each of their foals within the first 36 hours of birth. All samples were analyzed microscopically for piroplasms. Serum ELISA and PCR amplification of the 18S rRNA gene from DNA extracted from whole blood for *B. caballi* were conducted. Ninety (76.07%) mares and 40 (44.94%) of their foals were seropositive for *B. caballi*. Four (3.39%) mares and none of their foals were positive for *B. caballi* by PCR. Three out of the four PCR positive mares either had resorptions, abortions or stillbirths for that pregnancy.

Conclusions

This study indicates that transplacental transmission occurs only for *T. equi*.

0164

New date of spatial distribution of *Dermacentor reticulatus* tick in Romania

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Objectives

Dermacentor (D.) reticulatus (Fabricius, 1794) tick, the marsh tick or ornate dog tick is the second most significant tick disease vector in Europe. It is the vector of protozoae, rickettsiae and viruses. However, so far only very limited current information on the distribution of *D. reticulatus* in Romania is available. Few surveys on the geographical distribution of ticks in Romania were conducted in the 1960s and again in 2000s. No one of the 2000s studies however brought new data regarding the presence of *D. reticulatus* after Feider's early study from 1965.

Method

A new field survey monitoring the geographical distribution of *D. reticulatus* tick in Romania was carried out in 2012–2013 in order to compare the data on the distribution of *D. reticulatus* in Romania. In the present survey new areas with *D. reticulatus* occurrence were detected, providing evidence that this tick species has largely extended its geographical range or the tick species was not correctly identified in the past. The tick-host association of *D. reticulatus* is reported by the current study, showing new hosts. *D. reticulatus* is known to transmit *Babesia* spp. causing babesiosis in dogs and other tick borne-diseases.

Conclusions

The expansion of the geographical range of *D. reticulatus* in comparison to the data of the 1960s may therefore likely also cause a spread of canine babesiosis, which can be severe or fatal sometimes for dogs and of other pathogens transmitted by this tick vector.

0169

Some approaches to the seasonal dynamics of *Amblyomma cajennense* sensu lato in Villeta, Colombia

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Objectives

Ticks from the species *Amblyomma cajennense* s.l. (Acari: Ixodidae), are broadly distributed in America and its vectorial role for the transmission of *Rickettsia rickettsii* is recognized in different countries. Various studies in Colombia have identified the endemic status of Villeta town for Rocky Mountain Spotted Fever. The aim of this study was describe some aspects of the seasonal dynamics of *A. cajennense* s.l. in the rural zone from Villeta.

Method

Tick samples were collected between October 2012 and September 2013 from domestic animals (horses, cattle and dogs) and vegetation by direct collection and flagging and dragging, respectively. During the sampling, a total of 27762 ticks identified as *A. cajennense* s.l. were obtained. Predominant stages were larvae (51.6%) followed by nymphs (19.2%), males (17.9%) and females (11.3%). The mean number of larvae had a peak between June and August (2013); of nymphs between August and September (2013) and adults with two peaks, one in December (2012) and another between May and July (2013).

Conclusions

Herein, it is confirmed the ubiquity of this tick species in different domestic hosts and are illustrated some features of the dynamic of different stages in months were prophylactic measures could be introduced, in order to prevent the transmission of associated diseases to humans and animals. Further studies must confirm this seasonal patterns and its relation with the epidemiology of rickettsioses in the zone.

0183

Efficacy of different mosquito trapping methods in a humid area in Sicily

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Objectives

Culicidae, in particular *Culex pipiens*, are carriers of West-Nile virus, causing West-Nile encephalomyelitis. Virus reservoirs are wild birds and mosquitoes. In Sicily, Simeto Oasis, venue for nesting migratory birds, constitutes a risk area. Therefore a 2009-2013 monitoring plan has been activated performing monthly catches with different types of traps from March to October/November. In this study different capture methods were used and compared to assess their efficiency in adults culicidae catching.

Method

Three different types of traps were used: CDC light-trap with CO_2, BG Sentinel with CO_2 in combination with a chemical attractant (Lurex), Universal Trap with UV light and Lurex. Traps worked for 24 hours to capture both diurnal and nocturnal culicidae and mosquitoes were identified according to morphological keys. A total of 982 adult culicidae was caught by CDC light-trap. Out of these, 47% was identified as *Culex* spp., 37% as *Culex pipiens* and 16% as other species. Through the BG Sentinel, 1,038 mosquitoes were captured, out of which 58% was *Culex* spp., 34% *Culex pipiens* and the remaining 8% belonged to other species. Universal Traps allowed capturing 3,694 adult culicidae. Among these, 56% was identified as *Culex* spp., 39% as *Culex pipiens* and 5% as other species.

Conclusions

In this study a comparison among different traps and attractants efficiency was made. Universal Traps, although not foreseen in the plan, captured the greatest mosquitoes number, especially those with nocturnal habits such as *C. pipiens*.

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0187

Ticks and tick-borne pathogens in attractive tourist destinations of Croatia and Greece

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Objectives

Two expeditions to Croatia (May 2011) and Greece (May 2013), aimed at the mapping of the occurrence of ticks and tick-borne pathogens in the attractive tourist destinations were realized.

Method

In Croatia, more than 1700 ticks, belonging to five species have been collected. In the Plitvice Lakes National Park *Ixodes ricinus*, *Dermacentor reticulatus*, *D. marginatus* and *Haemaphysalis punctata* were recorded. In the forested Adriatic coastal zone (Makarska) only *Rhipicephalus sanguineus* ticks were present. Along the Greece coastline (Paralia district) more than 1000 *R. sanguineus* were collected from the vegetation and stray dogs. Sporadically, this tick species was observed in the strips of shrubs along the "wild" beaches. Isolated foci with a dozens of ticks per one m^2 were revealed in the neglected gardens with the occurrence of many guard and stray dogs near the coast and local roads. At the inland sites, in Meteora (central Greece) and in the Olympus National Park, up to an altitude of about 800 m a.s.l., the rare presence of *I. ricinus*, *R. turanicus* and occasionally also *H. inermis* and *D. reticulatus* was recorded. *Hyalomma marginatum* and *H. turanicum* were collected from the vegetation and caught turtles. Preliminary results indicate the presence of *Borrelia* spp., *Anaplasma* spp., and *Babesia* spp. in *I. ricinus* ticks from the Plitvice Lakes.

Conclusions

Results confirm that in the Mediterranean coastal areas, there are some places where the tourists and their pets may encounter ticks, especially at the beginning of the summer season.

The study was supported by the Research & Development Operational Program funded by the ERDF (ITMS 26220220116)

0196

Automatized analysis of behaviour activity with ticks (*Ixodes ricinus*)

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Objectives

Automated behaviour research is a worldwide increasing field of R&D. Mostly small animals as arthropods and rodents are used as target organisms in laboratory tests. To perform automated behaviour research studies, a range of different hard- and software providers are active on the market. This paper provides an insight in a behavioural study with ticks (*Ixodes ricinus*) with regard to a newly developed algorithm to solve a target-animal-size vs. survey-area-size problem which occurred with the used video tracking system (EthoVision XT 8.5, Noldus Information Technology).

Method

The ticks are placed in an area with a gradient of volatile molecules, as they are tested to act as attractants or repellents. The movement behaviour of the ticks has been tracked with a digital camera. The observed area was comparatively big (36 x 36cm) and the number of target organisms was high (up to 16 specimens), EthoVision XT 8.5 was not permanently able to properly recognise the ticks. This resulted in sudden losses of specimens during the tracking due to overlapping and leaps between individual animals. Additional problems occurred with random noise at the edges of the arena or due to dust particles or heterogeneous illumination. The recorded video data was transferred through the new algorithm which processed the digital data into binary images. A new algorithm was developed to follow automatically all individual ticks.

Conclusions

A video sequence of an experiment will be shown in a standard evaluation and analysed with the newly developed algorithm. It could be shown the even with small organisms as ticks (*Ixodes ricinus*) experimental designs in an observed area as 36x36cm are possible. The processed video data are free of leaps and overlapping errors and provides complete information about the movement of every individual tick.

0201

Blood parasites of the genus *Hepatozoon* found in snakes from Africa, America and Asia

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Objectives

The blood parasites from the genus Hepatozoon (Apicomplexa: Adeleida: Hepatozoidae) represent the most common intracellular protozoan parasites found in snakes. The species of the family Hepatozoonidae is transmitted by wide range of vectors include hard ticks, soft ticks and other arthropods.

Method

In the present study, we examined 209 individuals of snakes, from different zoogeographical regions (Africa, America, Asia and Europe), for the occurrence of blood parasites using both molecular and microscopic examination methods, and assess phylogenetic relationships of all *Hepatozoon* parasites from snakes for the first time. In total, 178 blood smears obtained from 209 individuals, representing 40 species, were examined, from which *Hepatozoon* unicellular parasites were found in 26 samples (14.6% prevalence). Out of 180 samples tested by molecular method polymerase chain reaction (PCR), the presence of parasites was observed in 21 individuals (prevalence 11.6%): 14 snakes from Africa belonging to six genera (*Dendroaspis, Dispholidus, Mehelya, Naja, Philothamnus* and *Python*), five snakes from Asia from the genus *Morelia* and two snakes from America, from two genera (*Coluber* and *Corallus*). The intensity of infection varied from one to 1433 infected cells per 10000 erythrocytes.

Conclusions

Results of phylogenetic analyses (Bayesian and Maximum Likelihood) revealed the existence of five haplotypes divided into four main lineages. The present data also indicate neither geographical pattern of studied *Hepatozoon* sp., nor congruency in the host association.

0205

The diversity of hard ticks (Ixodidae) in Slovakia

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Objectives

Slovakia represents the country where on relatively small area heterogeneous types of habitats are present in altitudes rising from 100 m to 2650 m asl. Due to global climate and social changes their distribution is changing and new infectious agents are emerging. The aim of our work was to map the occurrence of ticks in different climate model area of Slovakia. Study was realized in geographical temperature gradient, with respect to altitude in the natural environment.

Method

In total 6 exophilous epidemiologically important tick species were found questing on vegetation. Ixodes ricinus, the most abundant tick is present on the almost whole area of Slovakia. In last 2-3 decades it shifted its distribution from 600-800 m to higher altitudes and nowadays is present above 1200 m asl, constitution of vegetation cover is limiting factor. Two species of *Dermacentor* are present especially in the southern part. In warm and dry steppe areas *D. marginatus* is accompanied by Haemaphysalis inermis and in the wetland forests *D. reticulatus* was predominant species which is spreading along river flows by at least 200 km further and by 300 m of elevation into higher altitudes to Carpathians basins. It is accompanied by *H. concinna*. Occasionally *H. punctata* was detected.

Conclusions

Results of our study confirmed a presence of a wide spectrum of epidemiologically important tick species in Slovakia and revealed significant changes in their distribution.

0213

Soft ticks (Argasidae) on leptodactyloid frogs *Thoropa miliaris* (Spix, 1824) (Anura) in Brazil.

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Objectives

Soft ticks are ectoparasites of mammals, birds, reptiles and amphibians. Very little is known about soft ticks in amphibians. There are only two reports in the literature, one of them in Brazil, referring to *Ornithodoros* in *Thoropa miliaris* (Leptodactylidae), one leptodactyloid frogs endemic to the Atlantic Forest. This work report preliminary data of the parasitism by larvae of Argasidae in preserved specimens of *T. miliaris*.

Method

The analyzed specimens belong to the Herpetological Collection of the Federal Rural University of Rio de Janeiro, including extensive collections from 2002 to January 2014 on five islands and five locations on the mainland, in the state of Rio de Janeiro. The stage of the soft tick and the location of attachment were recorded on a frog scheme. From 147 *T. miliaris* examined 19 specimens were infested with larvae of soft tick (n=73). The prevalence, medium intensity, and parasite abundance were 12.92%, 3.84 and 49.61, respectively. On Islands the prevalence of ticks was 9.27%, while on the continent was 20%. Sixty-eight larvae of soft ticks were attached on both dorsal (legs and arms) and dorsolateral regions, and 5 ventrally.

Conclusions

It seems that the larval Argasidae has a place of predilection for attaching to *T. miliaris*, however this finding may be considered precipitated. It is noteworthy that others collections of *T. miliaris* in Brazil will be examined to determinate the real distribution of the parasitism. The specific identification of soft ticks and the study of host-parasite relationship focusing on island biogeography are in progress.

0220

Prevalence of *Anaplasma, Bartonella* and *Borrelia* species in *Haemaphysalis longicornis* collected from goats, Democratic People's Republic of Korea

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Objectives

The Democratic People's Republic of Korea (DPRK) is bordered by the Republic of Korea to the south, China and Russia to the north, and the Yellow and East Sea's on the west and east, respectively. While investigations of tick-borne disease surveillance have not been conducted in the DPRK, there have been numerous reports of tick-borne pathogens detected in ticks, associated zoonotic and domestic hosts, and humans in neighbouring countries.

Method

A total of 292 (27 nymphs, 265 adults) *Haemaphysalis longicornis* were collected from domestic goats at Rason, North Hamgyongbuk-do (Province), DPRK. Ticks were homogenized individually and assayed by nested-PCR using species-specific 16S or ITS primer sets. *Anaplasmabovis* (77/282, 27.3%) was the most frequently detected pathogen, followed by *Bartonella grahamii* (15, 5.3%), *A. phagocytophilum* (12, 4.3%), *Bartonella henselae* (10, 3.5%), and *Borrelia* spp. (3, 1.1%). Additionally, nested PCR using *gro*EL-based primer sets were used to obtain amino acid sequences of *B*. *grahamii* (6) and *B*. *henselae* (1). All gene sequences showed 100% similarity to each other and also showed close homology with sequences obtained from GenBank.

Conclusions

Tick-borne disease surveillance in the DPRK suggests that domestic animals may act as reservoirs for zoonotic tick-borne pathogens that impact on veterinary and human health. This report provides baseline data to better understand the ecology of tick-borne diseases in north-eastern Asia.

0231

Assessments of tick dominance in South African Nguni cattle

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Objectives

Ticks and tick-borne diseases are responsible for huge economic losses in beef cattle production systems in South Africa. These losses are due to the direct impact to the animals. In this study we assessed tick loads and species prevalence in Nguni cattle from three provinces of South Africa.

Method

Tick count data was collected over a period of twelve months in three farms (i.e. ARC Roodeplaat and Loskop farms and the Mukhutali Nguni Community farm).A total of 139 773 ticks (*Amblyomma hebraeum, Hyalomma marginatum, Rhipicephalus appendiculatus, Rhipicephalus (Boophilus) decoloratus* and *microplus,* and *Rhipicephalus evertsi evertsi*) were counted in those three provinces of South Africa. Location and month of tick counting had significant effect on the total tick count. ARC Roodeplaat and Loskop research farms had higher tick counts compared to Mukhutali Nguni Community farm. *Amblyomma hebraeum* followed by *Rhipicephalus evertsi evertsi* were the most dominant tick species in all three locations.

Conclusions

Further analysis with genetic marker data to develop a better understanding of tick tolerance in Nguni cattle is recommended.

0242

What are Portuguese ticks hiding?

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Objectives

Ticks are known as important vectors of etiological agents associated to human and animal disease. The "One Health" approach recognizes the need for integrated work of multidisciplinary teams as requisite for more comprehensive results in zoonotic diseases, such as tick borne diseases (TBD´s) which rely on animals as reservoirs. Tick identification and pathogen detection are critical in the analysis of disease risk and in the establishment of control measures. Thus, in this work it was our first concern to obtain data on the prevalence of ticks and tick-borne diseases such as anaplasmosis, babesiosis and theileriosis in Portugal mainland.

Method

Around 200 specimens were collected by flagging/dragging the vegetation and also from vertebrate hosts during 2013 in 32 randomly selected regional administrative regions of the country. Ticks were identified according to morphological characters and included species such as *Dermacentor marginatus, Hyalomma lusitanicum, Haemaphysalis punctata, Ixodes ricinus, Rhipicephalus bursa, R. pusillus* and *R. sanguineus*. Genomic DNA was extracted and tested for *Babesia* spp., *Theileria* spp. and *Anaplasma* spp. pathogens agents by conventional PCR.

Conclusions

Initial screening demonstrates presence of *Theileria* and *Anaplasma* pathogens in Portuguese ticks. The detection of agents in these arthropods reinforces the need of a continuing epidemiological survey.

0245

Surveillance for ticks and tick-borne pathogens in field and animal populations sharing Iberian lynx habitat

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Objectives

Iberian Lynx is one of the most endangered felids in the world, only occur in Spain and in Portugal. Because of its status as protect species, an epidemiological survey was carried out in potential lynx habitat. Ticks were collected from three areas in South of Portugal in order to address sanitary state and the circulation of tick-borne pathogens with potential impact on Iberian lynx.

Method

During 2012 and 2013, 167 ticks were collected either from vegetation or domestic *Felis catus domesticus* and wild animals, *Felis silvestris, Genetta genetta, Herpestes ichneumon, Martes foina, Vulpes vulpes*. Ticks were identified as *Dermacentor marginatus* (n= 12/7.2%), *Hyalomma lusitanicum* (n=114/68.3%), *Ixodes ricinus* (n=3/1.8 %), *Rhipicephalus bursa* (n=7/4.2%), and *R. pusillus* (n=21/12.6 %) and *R. sanguineus* (n= 10/6.0%). Molecular screenings performed to address the presence of tick-borne pathogens were directed to *Anaplasma spp., Babesia spp., Coxiella burnetii,* and *Theirelia spp.* DNA.

Conclusions

The results obtained provided information on tick-borne agents and reservoir ticks with potential impact on Iberian lynx populations and in their habitat.

0250

Prevalence of tick-borne pathogens in Northern Germany.

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Objectives

Although much effort is ongoing to study the risk of tick-borne pathogens in Germany, rather little is known about the prevalence in the North of the country. Therefore, around 1000 ticks have been collected during summer season in 2013 by flagging in different regions of Schleswig-Holstein and Mecklenburg-West Pomerania.

Method

Genomic DNA was isolated from single ticks. *Anaplasma phagocytophilum* (*Ap*) and the *Borrelia burgdorferi sensu lato* (*Bbsl*) group were detected in a Taqman-based multiplex real-time PCR modified after Courtney *et al*., 2004. For *C. burnetii* (Cb) two genes (*IS1111*, *com1*) were targeted in a multiplex real-time PCR after De Bruin *et al*., 2013.

The results showed that only *Ixodes ricinus* was found in the studied area. The preliminary analysis of the 984 ticks revealed that in total, 28.1% of the ticks were infected and the investigated pathogens were found in all tick stages. Among the infected ticks, 62.6% harbored a single pathogen (32.2% *Ap*, 29.7 *Bbsl*, 0.7% *Cb*) whereas 37.4% showed a multiple infection with more than one pathogen. Of these ticks (103), double co-infections (83.5% *Ap* and *Bbsl*, 1.9% *Cb* and *Ap,* 0% *Cb* and *Bbsl*) as well as triple co-infections (14.6% *Ap*, *Bbsl* and *Cb*) were observed. Interestingly, 78.9% of the *C. burnetii* positive ticks belonged to the triple infected samples.

Conclusions

Nearly 30% of the investigated ticks showed an infection with one or more of the human relevant pathogens *A. phagocytophylum*, *B. burgdorferi sensu lato* and *C. burnetii*. These results indicate that more attention has to be paid to the prevalence of tick-borne pathogens in the northern regions of Germany. Little is known whether co-infections cause supportive or competitive conditions for the pathogens. Nevertheless, the risk of multiple infections after tick bites has to be taken into consideration during medical care.

0264

Epidemiology of bluetongue virus in Mnisi, Mpumalanga

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Objectives

Bluetongue virus (BTV) is the aetiological agent of bluetongue (BT), a viral haemorrhagic disease that affects ruminant and camelid species. BTV is transmitted by biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae). The disease is of global economic concern due to its wide distribution and often high associated morbidity and mortality. The role of cattle in the epidemiology of BT in South Africa, as well as the distribution of different vector species throughout the country is not well understood.

Method

Mnisi, a rural area located in Mpumalanga, South Africa, was selected for an epidemiological study. The prevalence of Culicoides spp. associated with this area as well as whether BTV is circulating in the area were determined. Light traps were placed at four different sites in the Mnisi community during summer and winter periods. Additionally, sera were randomly collected from cattle (n = 1249) and screened with a BTV specific cELISA. Twenty-five different *Culicoides* species were identified of which *C. imicola* and *C. bolitinos* were found to be the most abundant even though the latter are usually associated with cooler regions of this country. Neutralizing antibodies were detected in serum of 1149 cattle, indicating that infection is highly prevalent, with a significant difference between age groups ($p<0.05$).

Conclusions

These results demonstrate that BTV as well as different vectors of the virus are circulating in the Mnisi area. The circulation of the virus between cattle and different midge species will assist in the understanding of the epidemiology of the disease.

0009

The character matrix approach to tick identification

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Objectives

An alternative to dichotomous keys for identifying a limited group of organisms uses character matrices. These sets of uniformly defined morphological features are used to define species when presented as full illustrations. Visual pattern matching correlates an examined specimen with a species description.

Method

For ticks a suitable group would be those of domestic animals within a defined region. In a matrix, row labels are morphological characters (e.g. 'density of punctations on scutum') together with two or more states for each character (e.g. 'sparse' or 'dense'). Column labels are the relevant species within one genus. Each cell is marked bimodally, positive or negative, for the state. Separately in a glossary all states of all characters are defined by sub-illustrations from the full illustrations together with a precise text definition.

Conclusions

The advantage of this system is the ability to enter the system at any point, quickly accessing the full range of character states relevant to one species. Identifications are supplemented by contextual information on distribution and hosts. This approach can be integrated with dichotomous keys if the same characters and states are used. The bimodal nature of character matrices is ideal for computer programs such as Multikey 2.1 for the ticks of domestic animals of Africa, which was named after its multiple entry system.

0039

Phylogeography of the Brown dog tick (*Rhipicephalus sanguineussensu lato*)

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Objectives

Brown dog ticks morphologically identifiable as *Rhipicephalus sanguineus sensu lato*, are distributed world-wide and their systematics is a subject of a controversy. Results of both a genetic comparison of geographically distinct populations of *R. sanguineus s.l.* and their reproductive compatibility indicate that *R. sanguineus* complex consists of at least two paraphyletic groups. To further elucidate systematic relationships within *R. sanguineus s.l.,* we conducted an extensive phylogeographical study of 90 tick specimens collected from dogs in 19 countries on the North and South American, Asian, and African continents.

Method

Voucher specimens were morphologically identified prior the genetic study. A phylogenetic tree was constructed using partial mitochondrial 12s rDNA, 16s rDNA and 18s rDNA gene sequences that were concatenated and analyzed by the Neighbour-Joining method. Three separate branches are clearly recognizable - *R. sanguineus* type 1, *R. sanguineus* type 2, and ticks closely related to *R. turanicus*. Each of these branches is as divergent as separate taxon. The greatest genetic diversity is found among specimens from the Mediterranean region (Israel, Iraq) suggesting that the divergence of *R. sanguineus* into distinct lineages may have initiated within this geographical region.

Conclusions

Results of this study are in agreement with the recently proposed division of *R. sanguineus s.l.* into tropical and temperate species. Based on these data, re-description and re-classification of brown dog ticks belonging to *R. sanguineus s. l.* is necessary.

0189

Description of nymphs of *Ornithodoros brasiliensis* Aragão, 1923 (Acari: Argasidae) based on optical and scanning electron microscopy

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Objectives

Ornithodoros brasiliensis is an endemic tick from Brazil. Its occurrence is restricted to the state of Rio Grande do Sul, southern Brazil. It is very aggressive to humans, causing fever, great pain and intense inflammatory response. After more than 50 years without report, the species was found in rural areas of São Francisco de Paula municipality, from where it was originally described. In 2012, its adult stage has been described however the description of the nymphal instars (N1- N5) has not yet been published. In the present study, we are describing all nymphal instars by optical and scanning electron microscopy.

Method

To the procedure, nymphs were selected from the laboratory colony maintained at Instituto Butantan. They were originated from specimens collected in rural areas of São Francisco de Paula municipality. Ten specimens of each instar were selected for morphological studies. Morphometric data were taken from 10 specimens of each instar. Five of those specimens of each nymphal instar were prepared for scanning electron microscopy. We observed that all the nymphal instars of *O. brasiliensis* morphologically resemble to the adult stage, differing in size and in presence of genital primordium.

Conclusions

The surface of tarsi flat dorsally and presence of dorsal humps, separate *O. brasiliensis* of the bat-associated species, as occurs with the others “ground ticks”.

0197

Genetic diversity of *Theileria equi18S rRNA* gene in horses from Rio de Janeiro, Brazil

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Objectives

The current study intended to define the phylogenetic position of *Theileria equi 18S rRNA* gene from samples collected in Rio de Janeiro, Brazil, comparing these to sequences deposited in the GenBank.

Method

Five equine DNA were used in a primary PCR (NBabesia1F; 18SRev-TB) to amplify the complete *T. equi 18S rRNA* gene. Three different PCR primer sets (NBabesia1F/18SRev-TB; NBabesia1F/BT18S3R; BT18S3F/18SRev-TB) were used in sequencing. Sequences were assembled and edited using DNA Baser 3.0. Sequence alignments were performed using ClustalW and adjusted manually (1,263bp). We include 36 sequences of *T. equi* available in the databases using BLASTN (*T. equi* from South Africa, Sudan, North America, Spain; *Babesia caballi* from South Africa; *Theileria parva* and *Theileria mutans* from South Africa. *Babesia bovis* were used as an outgroup. Neighbour-Joining was used to phylogenetic reconstruction and Kimura 2-Parameter to nucleotide substitution. Bootstrap analysis was used (1000 replicates) to test different phylogenetic reconstructions (MEGA5.1). All partial sequences of *T. equi18S rRNA* verified in BLASTN analysis yielded 99-100% similarity to previously deposited sequences. Analysis of *18S rRNA* sequences grouped the samples here studied into two major clades. The first clade was formed by KJ573371, KJ573372, KJ573374 sequences from this study and also the sequences from South Africa, Sudan and North America in Mexico border. The second clade was formed by KJ573370 and KJ573373 sequences and some minor sequences of South Africa, Sudan, further Spain and North America.

Conclusions

These results suggest a wide diversity among *T. equi* from Rio de Janeiro, Brazil.

0204

Free-living ixodid ticks in an urban Atlantic forest fragment, Brazil

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Objectives

As a consequence of the importance of ticks in forests in protected areas, was conducted survey of species of free-living ticks in the Natural Park Municipal Curió, state of Rio de Janeiro, Brazil.

Method

Monthly samples were taken by dragging method, dry ice traps and visual search in two transects. Adults and nymphs of *Amblyomma cajennense* (n= 147), *Amblyomma brasiliense* (n= 4) and *Amblyomma parvum* (n= 1) were collected. This is the first occurrence of *A. parvum* in the state. No correlation was found between the abundance of stages of *A. cajennense* and rainfall, temperature and relative humidity. The highest abundances of adults were in the months of January and May, and nymphs in September and October. The low diversity of parasites on Curió Park can be attributed to the proximity of households with pets, which would also explain the higher abundance of *A. cajennense* that is commonly found in areas impacted by anthropogenic pressure.

Conclusions

Tick sampling should be performed in Conservation Units, since they still preserve a structure of fauna and flora more closely resembling the natural environment of these arthropods, providing more information about their biology. In south-eastern Brazil, these areas correspond to Atlantic Forest remnants, which are favourable environments for ticks due to their abiotic and biotic characteristics, such as high percentages of relative humidity and moderate temperatures, allied to the presence of natural hosts.

0207

New records of *Ixodes* species collected in the municipalities of Cotia and Itapevi, State of São Paulo, Brazil.

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Objectives

Ticks of the genus *Ixodes* were collected from August 2011 to September 2013, in the Reserva Morro Grande and Condomínio Vila Verde, municipalities of Itapevi and Cotia, State of São Paulo, Brazil.

Method

The specimens were collected on vegetation, through cloth dragging and on rodents and marsupials captured in traps. Ticks were identified by morphological and molecular analysis. Two possible new species (ongoing study), and four others already described to Brazil were found. *Ixodes aragaoi* was collected on hosts and vegetation, while *I. fuscipes*, *I. cf lasallei*, *I. loricatus* and *I. schulzei* were found only on hosts, and *I. cf auritulus* on vegetation. In Reserva Morro Grande all of the six species were found, while in Condomínio Vila Verde only two. Merely *I. aragaoi* and *I. loricatus* had already been recorded to these localities. Among the six species collected, the most abundant were *I. loricatus*, *I. aragaoi* and *I. cf auritulus*, respectively. For the first time *I. schulzei* was collected in *Monodelphis americana* and *Delomys cf sublineatus*. Until now this tick species had already been recorded only in the rodents *Nectomys squamipes* and *Akodon montensis*. In Brazil, with the exception of *I. luciae*, there were no other records of *Ixodes* species collected by cloth dragging.

Conclusions

The high diversity of species found in a small area of Reserva Morro Grande, was an uncommon finding to this genus. These findings only reinforce the need for further studies and the little knowledge about the genus *Ixodes* in Brazil and in the Neotropical Region.

0211

Biology of *Amblyomma calcaratum* Neumann, 1899 (Acari: Ixodidae) in the laboratory.

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Objectives

The adult stage of *Amblyomma calcaratum* tick feeds almost exclusively on anteaters (Myrmecophaga tridactyla), while the few host records for immature stages were on Passeriformes birds. The present study reports biological data of two consecutive laboratory generations of *A. calcaratum*.

Method

Free-living developmental stages were observed in an incubator at 27°C and RH >85%. Immature feeding was observed at room temperature, using 6 chickens *Gallus gallus*, 6 Passeriformes birds *Serinus canaria*, and 6 wild mice *Calomys callosus*. Each host was infested with 1000 larvae or 28 nymphs approximately 25 days old. Two rabbits *Orytolagus cuniculus* were each infested by six pairs of adult ticks. The proportions of engorged larvae recovered per host species, mean larval feeding period in days, and mean larval pre-molt periods in days were: *G. gallus* (9.3%, 6.9, 23.9), *S. canaria* (17.2%, 7.4, 25.2), *C. callosus* (0.01%, 6, 21), respectively. Mean nymphal feeding period in days and nymphal recovery rates were: *G. gallus* (10, 0.5%), S. canaria (10.6, 23.2%), respectively. No engorged nymph was recovered from *C. callosus*. Mean nymphal pre-molt periods to males and females were 30 and 29.4 days, respectively. Twelve (100%) females were recovered from the two *O. cuniculus*, presenting the following mean values: feeding period-23.8 days, engorged weight-0.350g, pre-oviposition period-10.3 days, egg mass weight-0.140g, egg incubation period-52.2 days, Reproductive Efficiency Index (weight egg mass/female weight x 100) -29.7.

Conclusions

Our results corroborate the few available field data that indicate Passeriformes birds as main hosts for *A. calcaratum* immature stages.

0217

Molecular detection and characterisation of *Anaplasma* species in African buffalo (*Syncerus caffer*) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa

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Objectives

Bovine anaplasmosis, caused by *Anaplasma marginale*, is the most prevalent tick-borne disease of cattle worldwide and causes significant economic losses in the livestock industry. *Anaplasma marginale* subspecies *centrale* is a closely related species of low pathogenicity that is capable of inducing protection against *A. marginale*. The African buffalo is the natural reservoir host of various tick-borne haemoparasites of veterinary importance.

Method

In this study, the occurrence of *A. marginale* and *Anaplasma marginale* ss *centrale* in buffalo (n=91) from two geographically isolated national parks in South Africa (Kruger and Hluhluwe-iMfolozi) was determined using the reverse line blot (RLB) hybridization assay, quantitative real-time PCR (qPCR) and conventional PCR targeting four different genes (*msp1a*, *msp4*, *groEL* and 16SrRNA). RLB results indicated that 8% of samples were positive for the presence of *A. marginale* DNA, while 6% were positive for *A. marginale* ss *centrale* DNA. These samples also tested positive for *Ehrlichia*, *Theileria* and *Babesia* species. The qPCR assays indicated that 66% of the samples were positive for *A. marginale*, while 18% showed co-infection with both species. No *A. marginale* ss *centrale* single infections were detected. The PCR was used to amplify the four genes to generate sequence data and sequence differences will be discussed.

Conclusions

Our results indicate that *A. marginale* and *A. marginale* ss *centrale* are prevalent in African buffalo populations in South Africa which suggests that buffalo are natural reservoirs of *Anaplasma* species infection and could play an important role in the epidemiology and spread of anaplasmosis to the livestock industry.

NOTES:

Poster Session III

Friday 29th August (10h00-11h15)

0026

Field trial assessing deltamethrin (Butox®) treatment of sheep against *Culicoides* species

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Objectives

Culicoides (Diptera: Ceratopogonidae) biting midges may transmit various diseases of economic importance, including bluetongue (BTV) and Schmallenberg (SV) virus, which affect ruminants. During the outbreak of BTV in central and northern Europe in 2006, and in the absence of BTV vaccines, national veterinary services recommended the treatment of susceptible livestock with pyrethroids as a first-line defense against biting midges, although these insecticides were officially not registered and authorized for use against *Culicoides* midges.

Method

The efficacy of Butox® pour on (7.5mg deltamethrin/mL) against biting midges was evaluated in a double-blinded GCP field trial performed in Brandenburg, Northeastern Germany. Forty female Merino sheep with an average body weight of 38 kg (+/- 7 kg) were used for the study. Twenty randomly selected sheep were treated with 10mL Butox® pour on. The remaining 20 sheep were left untreated and served as a control group. Midge collections took place in two separate drop traps covering 2 crush pens with three confined treated/untreated sheep standing inside, on weekdays at 1, 7, 14, 21, 28 and 35 days post treatment. A total of 12.031 midges were collected inside the drop trap containing untreated sheep, in comparison to 7.026 midges collected from the vicinity of the treated sheep. Significantly more midges had fed on control compared to treated sheep, with 757 and 103 engorged midges respectively.

Conclusions

The results indicate that treatment of sheep with Butox® pour onprovided a significant decrease in *Culicoides* feeding rates under field conditions for at least 35 days.

0061

Mosquitocidal activity of a group of essential oils (monoterpenoids) against *Culex pipiens* L (Diptera, Culicidae) *In vitro* assessment

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Objectives

Mosquitoes are considered one of the most serious arthropod pests for humans. The female mosquito releases her saliva during feeding into animal blood, which may contain microorganisms responsible for some diseases, such as blue tongue, west Nile viruses and filariasis. The emergence of insecticide resistance has compromised the efficacy of insecticide-based control methodologies. Bioassays have revealed resistance of *C. pipiens* larvae to organophosphate insecticides in Egypt in three filariasis-endemic areas. The above in mind, the aim of this research was to evaluate the larvicidal activity of yeast-encapsulated citral, thymol and geraniol against 2nd, 3rd, and 4th instar larvae of *Culex pipiens.*

Method

Citral, Geraniol, Thymol - yeast shell encapsulated. Purified synthetic Citral, Geraniol, and Thymol (>98%) were purchased from Penta Chemical, (Edison, NJ) and absorbed into yeast cell shells (Biorigin, São Paulo, Brazil) to prepare a concentrate formulation containing 150 g/L yeast cell shells + 165 g/L monoterpenoids. The monoterpenoid formulations were provided by Eden Research, Plc (Oxfordshire, UK).Larvicidal activity of the encapsulated essential oil monoterpenoids was evaluated according to WHO protocols (2005). Based on wide range and narrow range tests, the concentrations used to test the activity of the encapsulated terpenes against second instar mosquito larvae were 0.22, 0.44, 0.88, 1.76 and 3.52 ppm (parts per million) while the concentrations used for the third and fourth instar were 0.69, 1.03, 1.54, 2.31, and 4.62 ppm. Encapsulated essential oil monoterpenoids were suspended in 1mL DMSO. After 20 seconds, third and fourth instar larvae were introduced into each solution. Larval mortality was recorded at 24 h and 48 h after initial exposure. The lethal concentrations (LC_{50} and LC_{95}) were calculated using the Data Processing System (DPS) computer program. Thymol showed the highest larvicide effect.

Conclusions

Targeting larvae is the most efficient method for controlling mosquitoes, such as *C. pipiens*, at their breeding sites. Our results indicated that all the encapsulated essential oil monoterpenoids tested have good larvicidal effect against *C. pipiens*. The tested concentrations of encapsulated Citral showed a good ability to control second and third instar larvae, with LC_{50} values of 1.45 and 2.03 ppm, respectively. Thymol was the most efficacious larvicidal essential oil monoterpenoid tested. Mortality of *C. pipiens* larvae exposed to the plant extracts increased with time of exposure and concentration, though this was not unexpected.

0152

Inhibition of egg laying and larval hatch in ixodid ticks treated with sub-lethal concentrations of flumethrin and flumethrin/imidacloprid combination

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Objectives

Ixodid ticks are important ectoparasites on farm animals and companion animals. They can cause direct effects in their hosts ranging from blood loss, toxin effects and general allergic reactions as well as secondary effects through transmission of tick-borne pathogens or bacterial infection. In addition to the direct control of tick infestations by compounds killing the parasitizing stages a reduction of the tick population can reduce the risk of tick infestation for all hosts. Tick-sterilizing effects have been observed earlier with cattle ticks (*Rhipicephalus microplus*) treated with sub-lethal doses of flumethrin.

Method

Recently, a flumethrin containing combination product has been introduced for use on companion animals. We have studied a potential population control effect of flumethrin and a flumethrin/imidacloprid combination on companion animal infesting ixodid ticks. Egg laying and egg development have been analysed after short term immersion of adult engorged females of relevant ixodid tick species. Under the conditions of this assay design no mortality of adults has been observed. Egg-laying has been partially or completely inhibited in a dose dependent manner. For the Brown Dog tick (*Rhipicephalus sanguineus*) 95% egg weight reduction has been observed at 1.31 ppm and 0.095 ppm for flumethrin and the flumethrin/imidacloprid combination, respectively. We will present comparative data on different fertility parameters from different ixodid tick species.

Conclusions

The results described are indicative of a potential population control effect of flumethrin containing products that provide additional benefit to the pronounced acaricidal effect of the compound.

0259

Assessment of genetic variability of chlorine channel-dependent glutamate: a susceptible and resistant strain to R*hipicephalus microplus* ivermectin

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Objectives

Chlorine dependent channels Glutamate is the site of action of the macrocyclic lactones such as ivermectin (IVM). In arthropods has been investigated that the mechanism of action of IVM is on the α subunit of the chlorine-selective channels, acting as agonists. Recently it has been reported that mutations in genes coding for the α subunit of GluCl channel. In *R. microplus* ticks, there are no reports or studies of these mutations found in the genes encoding the α subunit of GluCl channel and if they are associated with IVM resistance in field.

Method

The aim of this study was: To identify and characterize the chloride channel gen associated with ivermectin resistance in *R. microplus* tick. Field two strains resistant to ivermectin and other phenotypically susceptible when evaluated toxicologically were selected. Specific primers were designed using the sequence reported by Klafke (2010) in the Genbank amplicar PCR for the gene sequence. To extract the cDNA technique of phenol-chloroform was used and performed RT-PCR to amplify the gene. To visualize the amplicon gel was run at 1.8 %. Once amplified the sequence of interest is cloned in the Gateway technology using pENTRTM / D- TOPO vector, following the manufacturer's suggested method. The sequence obtained from the cloned strains was purified and sent for sequencing of genomic services section LANGEBIO. The sequences obtained were analyzed using the BLAST software and CLUSTALW.

Conclusions

Comparison of the sequences of the chloride channel in a susceptible strain resistant gene will identify possible mutations that may be associated with resistance.

0254

Bayesian prediction of *Amblyomma variegatum* dynamics using hidden process models

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Objectives

In silico evaluation of tick and TBD control practices requires a predictive dynamic framework that (1) approximates key density dependant / independent processes affecting tick numbers (2) captures the effects of external stochasticity (3) integrates prior knowledge (4) quantifies uncertainties in model choice, parameter estimates and predictions. Ecological time series are arguably the single most important data type for fitting and testing ecological forecasting models, yet, for want of a coherent methodological framework, fitting stochastic non-linear dynamic models to ecological time series whilst meeting these four requirements has long been an elusive goal.

Method

Two recently proposed algorithms, PMCMC [1] and SMC2 [2], could change this. These algorithms use particle filtering to fit non-linear stochastic hidden process models to time series and can, in theory, provide Bayesian inference for biological process models. But how much biological detail can be integrated into models under this paradigm and whether these algorithms really represent the state of the art in ecological forecasting remain open questions. We explore these questions by fitting population dynamic models containing various levels of biological detail to *A. variegatum* time series obtained from the 13 year Caribbean Amblyomma Program.

Conclusions

Ecological interactions are inherently non-linear and even the simplest non-linear systems can exhibit complex dynamics [3]. Identifying key sources of non-linearity is a fundamental pre-requisite for ecological forecasting. We explore whether simple non-linear models can characterize *A. variegatum* population dynamics using modern Bayesian methods. The relative advantages and disadvantages of the new methods and their implications for control program evaluation are discussed.

References

[1] Andrieu, Doucet, & Holenstein (2010) J. R. Statist. Soc. B, 72; [2] Chopin, Jacob & Papaspiliopoulos (2013) J. R. Statist. Soc. B, 75; [3] May (1976) Nature, 261.

0267

The presence of spirochetes from *Borrelia burgdorferi sensu lato* complex and tick-borne encephalitis virus in zoo animals in the Czech Republic

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Objectives

Ixodes ricinus, is the major vector of a variety of pathogens in Europe including, *Borrelia* spirochetes and tick-borne encephalitis virus. The ability of I. ricinus ticks to feed on a large variety of hosts (it parasitizes over 240 different vertebrate species) has important consequences for animal populations. Since the information on tick-borne infections in wildlife and exotic animals is very limited the monitoring of the presence of *Borrelia burgdorferi sensu lato* and tick-borne encephalitis virus in zoo animals in the Czech Republic, a country highly endemic for both of these pathogens, was performed.

Method

Serum from 69 species from 5 zoos located in different parts of the Czech Republic was collected for detection of antibodies against Borrelia and TBEV in zoo animals. Total 133 samples represented: even-toed ungulates (n = 78; 42 species), odd-toed ungulates (n = 32; 11 species), carnivores (n = 13; 9 species), primates (n = 2, 2 species), birds (n = 3; 2 species), and reptiles (n = 5; 3 species). The presence of antibodies against *B. burgdorferi s.l*. was investigated using the LYMETOP + Vet test (Promevet). The IMMUNOZYM FSME IgG all-species kit (Progen GmbH) was used for the detection of TBEV antibodies. Eighty samples were positive for Borrelia antibodies, including: samples from even-toed ungulates (n = 48; 28 species), odd-toed ungulates (n = 23; 10 species), carnivores (n = 7; 4 species), primates (n = 1; 1 species), and birds (n = 1; 1 species). All investigated reptiles (n = 5; 3 species) were negative. Only 2 individuals showed TBEV-specific antibodies: one markhor (*Capra falconeri*) and one reindeer (*Rangifer tarandus*).

Conclusions

While exposure of zoo animals to *B. burgdorferi s.l*. is common (60% of animals seropositive for *B. burgdorferi s.l*.), only 2 animals were seropositive for TBEV. This corresponds with data on prevalence of *B. burgdorferi s.l*. and TBEV in *I. ricinus* in Central Europe. Usually less than 1% of questing ticks are positive for TBEV, but 10–25% of ticks are positive for *B. burgdorferi s.l*. Our results indicate that a high number of animal species in the Czech zoos were exposed to *B. burgdorferi s.l*. and TBEV infection. Considering the pathogenic potential of both tick-borne pathogens, clinical and serological monitoring should be continued.

0176

First report of the isolation and Molecular Characterization of *Rickettsia amblyommii* from *Amblyomma cajennese* sensu stricto in Maranhão, northeastern Brazil

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Objectives

The spotted fever group agent, Rickettsia amblyommii, has been reported infecting *Amblyomma* ticks in the United States, Costa Rica, Panama, French Guiana, Brazil, Paraguay and Argentina. In Brazil, *R. amblyommii*-infected ticks have been reported in five biomes, namely Amazon, Caatinga, Savannah, Atlantic Rainforest and Pantanal. Furthermore, there is serological evidence for human and canine infection by this rickettsia. The *Amblyomma cajennense* complex (Fabricius, 1787) is composed by six species that feed on a variety of vertebrate hosts, mostly mammals.

Method

In this study, ticks were collected from free-ranging domestic pigs in Viana, Baixada Maranhense region during rainy (March) and drought (September) periods of 2013. Collected ticks were identified as *Amblyomma cajennese* sensu stricto (64 males, 19 females, and 10 engorged nymphs that molted to 4 males and 6 females in the laboratory). A total of 57 ticks were tested individually for presence of *Rickettsia* by PCR assays targeting two rickettsial genes (*gltA* and *ompA*); some of these ticks were also processed for isolation of rickettsiae in Vero cell culture through the shell vial technique. Overall, 8 (14.0%) ticks yielded *gltA* and *ompA* amplicons, which generated DNA sequences 100% identical to *Rickettsia amblyommii* from GenBank. Isolates of *R. amblyommii* were obtained in Vero cell cultures from 2 of the PCR-positive ticks.

Conclusions

These results expand the distribution of both *Amblyomma cajennense* sensu stricto and *R. amblyommii* in South America.

0268

Transmission dynamics of *Borrelia* bacteria in a bird tick community

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Objectives

We examined the *Borrelia burgdorferi s.l.* circulation in a tick community consisting of three species (*Ixodes ricinus*, *I. frontalis*, *I. arboricola*) with contrasting ecologies, but sharing a common host: the great tit (*Parus major*), one of the most common birds of European gardens and woodlands.

Method

Field data show that the birds hosted Borrelia-infected larvae of both *I. frontalis* and *I. ricinus*, indicating the facilitation of Borrelia transmission. The low, but significant numbers of Borrelia in unfed *I. arboricola* ticks collected from bird nest boxes, provide the first evidence that it is competent in maintaining Borrelia over long periods of time. Aside from the known avian genospecies (*B. garinii* and *B. valaisiana*), several less dominant genospecies were observed in the three ticks, including *B. turdi* and some mammalian genospecies. In laboratory experiments, we imitated the natural situation during the bird's post-fledging period, in which Borrelia-naïve juvenile birds are repeatedly exposed to infected *I. ricinus* nymphs. Birds developed systemic infections of the avian genospecies. Although birds showed a very low competence to facilitate the transmission of mammalian genospecies, a low number of birds remained permissive for *B. afzelii*. Infected birds were able to transmit Borrelia to naïve *I. frontalis* and *I. arboricola* individuals. However, the latter tick species were not able to transmit the bacteria to a new host.

Conclusions

When using the great tit as a host, transmission cycles are driven by *I. ricinus*, and are not maintained by the ornithophilic ticks (*I. frontalis and I. arboricola*). However, spill-over of the bacteria from *I. ricinus* to the ornithophilic tick species often occurs in the wild. The use of bird species, other than the great tit (e.g. thrushes and finches), may result in different transmission outcomes than reported here.

0273

Attempted infection of common waterbuck (*Kobus ellipsiprymnus*) with buffalo-derived *Theileria parva*

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Objectives

Following reports that defassa waterbuck (*Kobus defassa*) may play a role in the epidemiology of *T. parva*-group infections in cattle in East Africa, common waterbuck (*Kobus ellipsiprymnus*) in Kruger National Park (KNP) were investigated as potential carriers of *T. parva*-group infections.

Method

Seven adult and sub-adult common waterbuck were captured and screened by conventional and molecular diagnostic techniques for Theileria spp. infections. Laboratory-reared *Rhipicephalus zambeziensis* nymphs were fed in ear bags on 4 captive buffalo (*Syncerus caffer*) confirmed to be naturally infected with *T. parva*. The resultant adult ticks were fed on 4 captive sub-adult waterbuck and 2 cattle. All the waterbuck were found to carry microscopically detectable Theileria sp. piroplasm infections, found by PCR diagnosis to belong to a hitherto uncharacterised Theileria species. *R. zambeziensis* adults which fed as nymphs on the buffalo transmitted fatal *T. parva* infections to cattle. However, no transmission of T. parva to the waterbuck could be demonstrated clinically or by PCR diagnosis. Also, *R. zambeziensis* nymphs subsequently fed on the waterbuck failed to transmit *T. parva* to cattle in the resultant adult stage, confirming the absence of *T. parva*-group infections in the waterbuck.

Conclusions

The results suggest that buffalo in KNP probably do not carry *T. parva*-group parasites which are readily transmissible to common waterbuck which are therefore unlikely to play an important role in the epidemiology of *T. parva*-group infections in cattle in South Africa.

0004

Onset and duration of immunity in dogs after administration of the preparation based on FERRITIN 2 recombinant protein

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Objectives

Ferritin 2 in the tick's body acts as a transporter of iron, which tick receives from the blood. If Ferritin 2 is blocked, the tick is not able to parasitize on the host, because mechanism of iron transfer is not functional. The ticks sucking the blood in the vaccinated animals suck less time and the risk of transmission of the tick-borne diseases is lower. This fact makes Ferritin 2 ideal candidate for the vaccine. Recombinant *I. ricinus* (IrFER2) proteins were expressed in *Escherichia coli*, used for immunization of dogs and antibody levels were monitored.

Method

In the process of testing of antibody response 10 dogs were vaccinated subcutaneously with FERRITIN 2 antigen 100 µg per vaccination dose 1.0 ml. PET GEL A (Seppic) was used as an immune adjuvant. Four dogs were used as the non-vaccinated controls (PLACEBO). In the vaccinated group 5 dogs were vaccinated with 3 doses (vaccination schedule A) and 5 dogs were vaccinated with 2 doses (vaccination schedule B) at intervals of 21 days. All animals were re-immunized approximately 1 year after the primary immunization. The blood samples were taken in predetermined intervals, sera samples were prepared and these were tested by in house indirect sandwich ELISA method. Post-vaccination antibodies against FERRITIN 2 were detected using the ELISA sets with highly purified specific recombinant protein FERRITIN 2. Antibody levels in the tested sera were expressed as an OD_{450} values, compared to the values of the control positive and negative sera. The immunisation schedule A (vaccination + 2 revaccinations) induced better antibody response and longer persistence of antibody levels in comparison with schedule B. Antibody levels declined gradually, slightly below the value of the control positive serum since 250th day of testing. Each additional immunization, including annual revaccination induced significant booster effect.

Conclusions

Immunisation of dogs with recombinant FERRITIN 2 protein in conjunction with suitable immune adjuvant induces high specific antibody levels in sera. Vaccination schedule involving primary vaccination + 2 revaccinations is better for longer persistence of antibodies. ELISA examination enables determination of specific post-vaccination antibodies against Ferritin 2. Detection of these antibodies and their quantification may be used for evaluation of efficiency of vaccines and onset and duration of immunity.

This work was supported by Project FR-TI3/156 from Ministry of Industry and Trade, CR.

0006

Testing of activity of the growth-inhibiting antibodies using in vitro growth inhibition test.

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Objectives

The existing problems in the diagnosis and treatment of Lyme disease gave rise to an urgent need to manufacture a vaccine that would be capable to effectively immunize susceptible species of domestic animals, especially dogs, against infection with *Borrelia burgdorferi* sensu lato. Protective efficiency against borrelia antigen contained in a vaccine must be proved by a challenge test on the target species before registration. Infection of the target species has to be performed via infected ticks. Implementation of suitable routine in vitro method for determination of presence of protective antibodies could replace the challenge tests in the target animals.

Method

The sera samples from the dogs vaccinated with whole cells *Borrelia burgorferi* sensu stricto, *Borrelia afzelii* and *Borrelia garinii* antigens were tested for their capability to inhibit the growth of borrelia in vitro. Sera from the non-vaccinated dogs were used as a negative control. These sera were tested for presence or absence of the anti OspA antibodies first. Different borrelia strains were used in these tests. The tests were performed in flat-bottom 96-well microtiter plates. Sterile filtered and heat inactivated sera samples were serially diluted in BSK II containing phenol red, live borrelia cultures and guinea pig complement were added to each well and incubated at 33 °C for 8 days. During incubation, the absorbance was measured. Bacterial growth was measured as a function of the pH, indicated by a colour change in the medium from red to yellow. The sera from the vaccinated dogs showed presence of the growth-inhibiting antibodies detectable by this test. The sera from the non-vaccinated dogs contained minimal or no growth-inhibiting antibodies, but if the complement guinea pig dependent strain of borrelia was used, false positive results were observed, as if high titres of growth-inhibiting antibodies in the negative sera were detected.

Conclusions

The growth inhibition test (GIT) appears to be a suitable method for the detection of protective antibodies against borrelia infection. The critical point of this test is selection of suitable, guinea pig complement resistant borrelia strain. Validation of the GIT is necessary for its using as a method of efficacy testing of vaccines against Lyme disease. This work relates to the proposal of the test model of efficacy of the vaccines against Lyme disease and the creation of the draft of the Pharmacopoeia monograph.

This work was supported by Project FR-TI4/092 from the Ministry of Industry and Trade, Czech Republic.

0032

Salivary gland transcriptome of female *Rhipicephalus (Boophilus) decoloratus*

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Objectives

Ticks and tick-borne diseases are widely distributed globally impacting on human and animal health, and regarded as a major cause of poverty in most of the African continent. Tick control using chemicals was first introduced in South Africa and has been practiced for over 100 years. The chemical control has major disadvantages as environmental pollutants and resistance to most acaricides is a constraint to control policies. New immunological approaches targeting tick feeding mechanisms for control purposes should be considered. *Rhipicephalus (Boophilus) decoloratus* has a wide distribution in Africa and vector of *Babesia bigemina*, *Anaplasma centrale* and *Anaplasma marginale*.

Method

Next-Generation sequencing technology was used for the construction and sequencing of female *R. (B.) decoloratus* salivary gland cDNA libraries from different feeding stages. Assembly, analysis and annotation of the transcripts were performed using CLC Genomics Workbench. Different feeding stages showed differential expression. Comparison of putative secreted and housekeeping transcripts with sequences in the database indicated that the salivary gland transcriptome of *R. (B.) decoloratus* is similar to other metastriate transcriptomes sequenced to date. Selected protein candidates will be expressed and functionally characterized and proteomics analysis will be used to validate and confirm the transcriptome.

Conclusions

Salivary gland transcriptome of *R. (B.) decoloratus* is similar to other metastriate transcriptomes sequenced to date. Comparative data on the salivary gland transcriptome of R. (B.) decoloratus remains unexplored and attaining transcripts of sufficient coverage and quality for different feeding stages will facilitate the discovery of new anti-tick vaccine candidates and serve as a model for the understanding of the evolution of blood-feeding mechanism in ticks. This study could add to the already existing tick genome database, and identification with the potential to be used as vaccine candidates.

0076

The isolation and identification of potential vaccine antigens against poultry red mite

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Objectives

The poultry red mite is the most economically important ectoparasite affecting laying hens throughout the world. Bird welfare suffers due to red mite blood feeding. Current acaricidal control is not sufficiently effective due to increased resistance thus alternative control strategies are required.

We aim to isolate concealed antigens from the gut of mites and test their efficaciousness as potential vaccine candidates. Effective natural immunity has been previously demonstrated from the tick vaccine TickGARD™ using concealed antigens against the cattle tick *Rhipicephalus (Boophilus) microplus* thus we attempt to translate this concept towards the control of poultry red mite.

Method

Current protocols are based on separating membrane proteins via low speed differential centrifugation. This has provided distinct protein fractions validated via western blots with already defined anti-Cathepsin D and Histamine Release Factor antibodies. Mass spectrometry and comparison of these samples to our red mite transcriptome library has revealed several red mite proteins of interest. Antibody libraries specific to these isolated proteins will be created by biopanning and should help to identify the location of the expression of our potential vaccine targets via immunohistochemistry. An in-house mite collection and isolation protocol has been established and a protocol for sectioning mites is also showing early promise to be used for our future studies.

Conclusions

The project is on track during the second year of the PhD and has developed initial interesting results and a good foundation of standardised methods for studying red mites here at the RVC.

0105

Effects of recombinant Subolesin vaccine on tick infestations in cattle and sheep farms

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Objectives

Several species of ticks are associated with the transmission of diseases of great impact to the human and animal health and are the most important vectors of pathogens affecting livestock. Despite the use of chemical acaricides, tick infestations continue to affect livestock worldwide. Tick vaccines have been proposed as a cost-effective and environmentally friendly alternative for tick control. This study was aimed to investigate the effect of vaccination with the candidate tick protective antigen, Subolesin, on tick infestations in both cattle and sheep livestock.

Method

Two cattle and two sheep farms with similar geographical locations and production characteristics were randomly assigned to control and vaccinated groups. All the animals of the vaccinated groups were immunized with two doses of 1 ml containing 100 µg of the Subolesin fused to *Anaplasma marginale* Major Surface Protein 1a. Serum antibody titers were determined using antigen specific indirect ELISAs against Subolesin. In these farms ticks were collected, counted, weighed and classified for one year prior to and 9 months after vaccination. An antibody response against Subolesin following vaccination was observed in both farms. The percent of infested cattle was reduced by 8-fold, while in sheep a reduction of tick infestations by 63% was observed. A decrease in female tick weight by 32-55% was observed in ticks collected from both vaccinated cattle and sheep when compared to controls.

Conclusions

These results support the efficacy of recombinant vaccines in controlling tick infestations, encouraging their use for tick control in cattle and sheep, even under multiple tick species infestations.

0106

Ticks with long hypostome induce morphological changes in different cell lines.

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Objectives

During feeding, the tick's hypostome penetrates into the host skin, damages it and comes in contact with both keratinocytes and fibroblasts. Skin injury activates repair processes requiring interactions and communications between a variety of cells and involving various growth factors, cytokines and other soluble factors. However, bioactive molecules in tick saliva manipulate host immune responses. In a previous study, we demonstrated anti-growth factors activities in salivary gland extracts (SGE) from different tick species. To investigate whether these SGE activities have an effect on cells, we examined SGE derived from various ticks on cell proliferation, morphology and cell cytoskeleton.

Method

For proliferation assay, the cell lines were selected based on a) the assumption of their reflection of properties of cells in the skin (keratinocytes, fibroblasts) and b) their intrinsic medicinal interest (glioma cells). SGE derived from tick species with long hypostome – *Amblyomma variegatum, Hyalomma excavatum* and *Ixodes ricinus* – inhibited cell proliferation *in vitro* and induced changes in the morphology of different cell lines. These effects correlated with disruption of the actin cytoskeleton and could be related to ability of these ticks bind platelet-derived growth factor (PDGF) described previously. Such effects were not observed with SGE from ticks with short hypostome and not targeting PDGF – *Dermacentor reticulatus* and *Rhipicephalus appendiculatus*.

Conclusions

Although PDGFs are known to be involved in cellular proliferation through their receptors, which also stimulate rearrangement of actin filaments, there is as yet unknown, whether the *in vitro* effects on cells of SGE from ticks with long hypostome are due to anti-PDGF activity.

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0107

Long mouthparts ticks versus short mouthparts ticks in anti-growth factors activities

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Objectives

The 'bite'of ixodid ticks and their protracted feeding periods evoke host wound healing and immune responses. Ticks must suppress skin immune reactions to complete feeding and development. The signalling network involved in regulation of the wound healing process includes cytokines, chemokines and numerous growth factors, including epidermal growth factor (EGF), fibroblast growth factor (FGF-2), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-b1), granulocyte - macrophage colony stimulating factor (GM-CSF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF).

Method

Used ELISA method, chemical cross-linking assay with radiolabeled growth factor we demonstrated that constituents of tick salivary gland extracts (SGE) bind growth factors: TGF-b1, PDGF, FGF-2, and HGF, but not EGF, VEGF and GM-CSF, depending on tick species. SGE derived from *Amblyomma variegatum* and *Hyalomma excavatum* reacted with TGF-b1, PDGF, FGF-2 and HGF; *Dermacentor reticulatus* and *Rhipicephalus appendiculatus* with TGF-b1, FGF-2 and HGF; and *Ixodes ricinus* and *Ixodes scapularis* with PDGF.

Conclusions

The process of wound healing depends on wound depth: a deeper wound of epidermis and dermis requires a longer, more complex process of healing. Our results support the hypothesis that the complexity of modulation of host immune responses by ticks correlates with the length of their hypostome.

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0112

Characterisation of a lipocalin-like protein from *Ornithodoros savignyi*

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Objectives

Ticks have developed many strategies to overcome the protective mechanisms of the host which they face during a blood meal. Lipocalins proteins are abundantly expressed in the salivary glands of both hard and soft ticks and are well recognised as scavengers of hydrophobic molecules and antagonistic agents that target host proteins and receptors.

Method

While attempting to identify tick proteins that recognize and bind to Gram-negative bacteria, a lipocalin-like transcript was identified in the hemolymph of the soft tick *Ornithodoros savignyi*. The mature protein of 188 amino acids has a predicted molecular mass of 21 481.9 Da and pI of 4.37. mRNA expression profiling indicates up regulation in hemolymph upon hemocoelic bacterial challenge and feeding, suggesting a possible antimicrobial role. Although no up-regulation was detected in the midgut or ovaries, it appears to be constitutively expressed and may therefore be related to post-feeding development such as blood digestion or egg production. To date, all tick lipocalins described are salivary gland-derived and since no transcription was detected in the salivary glands, it is possible that the expressed functional protein (savicalin) may play a larger role in tick biology that is not restricted to feeding alone. Savicalin was cloned and recombinantly expressed using a variety of vectors. Further novel improvements to the expression and purification methods resulted in soluble protein more suitable for biochemical and biophysical characterization.

Conclusions

Studies regarding its role as an antimicrobial and immune modulator are currently being carried out in order to understand its significance in tick immunity.

0113

Effects of immunization with the tick-derived AvPDI protein on feeding and metamorphosis of different tick species and transmission of *Borrelia afzelii* by *Ixodes ricinus*

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Objectives

Because of the increasing incidence of tick-borne diseases over the past few years, tick control becomes critical. Anti-tick vaccines represent a promising alternative to chemical control. Identification of antigens that evoke host immune response and block early phases of tick feeding is a major research goal in development of anti-tick vaccines and vaccines blocking transmission of tick-borne pathogens. A potential candidate for such vaccines is the AvPDI protein derived from tissues of *Amblyomma variegatum* ticks.

Method

We investigated the effects of immunization of laboratory mice with recombinant N-terminal AvD-GST fusion protein on feeding success, weight, metamorphosis and oviposition of *Ixodes ricinus, Amblyomma variegatum* and *Rhipicephalus appendiculatus* ticks. In addition, potential transmission blocking effects of AvD-GST were studied on the mouse - *I. ricinus* - *Borelia afzelii* model. Despite strong anti-AvD-GST antibody response in mice, we did not detect any significant effects of immunisation on feeding success, development/metamorphosis or oviposition of the studied tick species. Immunisation with AvD-GST did not impair transmission of *B. afzelii* spirochaetes via infected *I. ricinus* nymphs to mice nor to nymphs subsequently feeding on hosts infested primarily with *Borrelia*-infected ticks.

Conclusions

Further studies are needed to elucidate the role of different AvPDI variants in tick feeding and pathogen transmission, involving other animal models, tick species and pathogens.

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0120

Perforin and CD25 expression in *Theileria parva* specific CTL correlate with cytotoxicity

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Objectives

Theileria parva, a protozoan parasite, the causative agent of the cattle disease East Coast fever is responsible for major economical loses in Sub-Saharan Africa. In the effort of generating a second generation vaccine against ECF, we have undertaken a study to identify immunological markers that correlate with T cell cytotoxicity, which is known to be important for protection against the disease.

Method

We examined if the number of tetramer positive CD8 cells and the staining intensity of these correlated with the cytotoxicity of the target cells. Furthermore, we investigated if the expression of two activation markers, perforin and CD25 correlated with the cytotoxicity. Bulk CTL lines and purified CD8 cell lines generated from cattle of the A18 BoLA (MHC) type were analysed for the *Theileria parva* specific immune responses using a peptide-MHC tetramer and antibodies for perforin and CD25 in FACS analysis. Cytotoxicity was measured using Cr-51 release assay. The results demonstrate that the percentage of tetramer positive cells in six cell lines correlate with killing of PBMC pulsed with the peptide. A better correlate with cytotoxicity was obtained using the product of the percentage tetramer positive cells and the mean staining intensity (MFI). Likewise, the product of percentage perforin positive cells and the staining intensity had the best significant correlation with killing of the pulsed PBMC. The MFI of CD25 positive tetramer positive cells correlated negatively with the cytotoxicity. These results suggest that perforin and CD25 could be possible biomarkers for the cytotoxicity to *Theileria parva* infections/immunizations.

Conclusions

In conclusion, perforin and CD25 may be possible biomarkers for T cell cytotoxicity.

0123

Evaluating cell surface display as a potential brucellosis antigen delivery system

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Objectives

Brucellosis is a global zoonotic disease, associated with significant morbidity that can lead to spontaneous abortion and infertility in livestock. *Brucella abortus* is the causal agent of bovine brucellosis. Current vaccines against bovine brucellosis are based on live attenuated strains of *Brucella*. The disadvantages associated with the use of these vaccines include being infectious to humans, interference with diagnosis as they elicit similar immune profiles to infected animals, and induction of abortions in pregnant animals.

Method

CSIR Biosciences has developed *Yarrowia lipolytica* as a potential cell surface display host. Using the above-mentioned display system and the *Escherichia coli* outer membrane protein (OmpA) and auto-transporter (AIDA-I) display systems, *Brucella* antigens derived by reverse vaccinology will be surface displayed for the development of a viable, recombinant whole cell brucellosis vaccine. A web-based vaccine design program (Vaxign) was utilized to predict candidate vaccine targets based on adhesion, epitope binding to MHC class I, displaying no homology to humans, mice and pig proteins and subcellular localization to the outer membrane.

Conclusions

Using these criteria, the outer membrane proteins, Omp16 and Omp19 were selected as targets. *In silico* peptide prediction (IEDB and Epitopia servers) was utilized thereafter, to select regions in the Omp16 and Omp19 amino acid sequences that have antigenic (B cell and T cell epitopes) traits. Furthermore, Omp16 and Omp19 protein modelling was performed to characterize surface exposed loop regions. The characterized surface exposed and antigenic epitopes will be displayed on the surface of *E. coli* using OmpA as the anchor protein.

0127

Changes in global gene expression in brains of mice with different clinical course of tick-borne encephalitis

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Objectives

Tick-borne encephalitis (TBE) is caused by one of the most prevalent arboviruses in Europe and in north-eastern Asia, tick-borne encephalitis virus. Clinical course of TBE ranges from asymptomatic or mild influenza-like infection to severe debilitating encephalitis or encephalomyelitis. Serious clinical symptoms result from TBEV propagation in the brain. Only little attention has been paid to the underlying genetic determination involved in disease severity. To address this critical issue we developed an animal model of TBE based on BALB/c-c-STS/A (CcS/Dem) recombinant congenic mouse strains showing different severities of the infection in relation to the host genetic background.

Method

We have compared genome-wide profiles of gene expression in brains after of uninfected and TBE virus infected mice of strains BALB/c, STS and CcS-11 (strains that exhibit different clinical course of the infection with TBE virus) using GeneChip® Mouse Gene 2.0 ST Array (Affymetrix). Our results revealed different expression level in wide range of immunologically important genes (in particular, up-regulation of: TLR-3 gene expression for STS strain; histocompatibility 2, D region locus, 2'-5' oligoadenylate synthetase-like 1 and IL1β for BALB/c strain), that are associated with the development of inflammation in the brain.

Conclusions

Taken together with our previous results these data highlight and extend our conclusions indicating that different seriousness of tick-borne encephalitis in different mouse strains is associated with different changes in global gene expression in brains. These results are underlying importance of genetic determination involved in determining the outcome of TBE virus infection, which has crucial consequences to future development of rational therapy of TBE.

0132

The development of live attenuated tissue culture vaccine against heartwater in South Africa

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Objectives

Heartwater is an infectious tick-borne disease of ruminants caused by the rickettsia, *Ehrlichia ruminantium* which, in South Africa, is transmitted by *Amblyomma hebraeum.* The disease is regarded as one of the most economically important livestock diseases in the country as it affects cattle, sheep, goats and wild ruminants.

Method

Intravenous and intramuscular inoculation of the live attenuated tissue culture vaccine into sheep, cattle and goats and subsequent needle challenge with the virulent strain or natural field challenge. Results look promising.

Conclusions

Experiments are in progress to determine the optimal effective immunizing dose, reversion to virulence, up-scaling of the vaccine, shelf life and cold chain stability.

0148

Effective immunogenic chemical conjugations for P0 antigen in dogs against Brazilian *Rhipicephalus sanguineus* ticks

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Objectives

Recently, the high effectivity against *Rhipicephalus sanguineus* ticks of one peptide in the ribosomal protein P0 has been reported in rabbits. This study was designed to demonstrate whether this peptide in different formulations used to immunize dogs, the natural host of *R. sanguineus* ticks, affects the biotic potential of a Brazilian stock of these ectoparasites.

Method

Chemical conjugates of P0 peptide (pP0) with hemocyanin of *Megatula crenulata* (KLH) or Bm86, the active principle of GAVAC, as carrier proteins were prepared with VG Montanide 888 oily and used to immunize Beagle dogs. Fifteen days after the last immunization, dogs were challenged with all stages of R. sanguineus ticks. Dogs vaccinated with both chemical conjugates developed effective antibody titers against pP0. Dogs vaccinated with the pP0-Bm86 conjugate also developed antibody titers against Bm86 antigen. After challenge, a significant high mortality in all tick stages was observed in vaccinated groups with conjugates. In addition, the recovered alive specimens on the vaccinated groups with conjugates weighed less that the recovered specimens on the control group.

Conclusions

These results show the potential of this peptide to develop an anti tick vaccine.

0154

Evaluation in BALB/c mice of the vaccine of *Anaplasma marginale* produced in IDE8 cells associated with rMSP1*a*, using carbon nanotubes as a carrier molecule.

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Objectives

The immunogenic potential of MSP1*a* and its role in the development and transmission of *Anaplasma marginale* makes this protein a vaccine candidate for bovine anaplasmosis. The carbon nanotubes revealed new perspectives for their potential applications in biological fields. The objective of this study was to evaluate the immune responses of BALB/c mice immunized with the vaccine of *A. marginale* produced in IDE8 cells associated with rMSP1*a*, using carbon nanotubes as a carrier molecule

Method

The fragment of rMSP1*a* comprising the N-terminal region of the protein was expressed in *Escherichia coli* BL21, purified and covalently linked to multiwalled carbon nanotubes (MWNTs). Thirty BALB/c mice were divided into five groups and immunized subcutaneously at days 0, 21 and 42: G1 (rMSP1*a*), G2 (MWNT+rMSP1*a*), G3 (MWNT), G4 (vaccine produced *in vitro* associated with MWNT+rMSP1*a*) and G5 (adjuvant). Blood samples were collected on day 11 after each immunization to evaluation of anti-rMSP1*a* IgG response by ELISA. The spleens were collected and the splenocytes were cultured for cell proliferation assays and cell immunophenotyping. Mice immunized with rMSP1*a* (G1, G2 and G4) produced high levels of anti-rMSP1*a* IgG. However, G4 group showed lower levels anti-rMSP1*a* IgG than G1 and G2 groups. Immunization with MWNT+rMSP1*a* (G2 and G4 groups) significantly induced higher percentages of $CD4^+CD44^+$ and $CD4^+CD62L^+$ lymphocytes, high levels of TNF-α,and a higher proliferative rate of splenocytes compared to mice from G1 group.

Conclusions

Therefore, additional experiments using cattle should be performed to determine the efficacy, safety, immunogenicity and protection induced by rMSP1*a* associated with MWNT.

0162

Intestinal damages in ticks feeding on immunized cattle with a subunit vaccine against *Rhipicephalus microplus*

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Objectives

Rhipicephalus microplus is of greatest economic impact on cattle livestock, the control are based on acaricides, which are contaminants of environment and too animal source foods, the development of alternative methodologies aroused interest. The, immunological has become an option. Studies covering histological changes in the midgut after immunization with recombinant and synthetic peptides as vaccines in cattle have not been done. Here are described the histological changes induced in *R. microplus* exposed to bovine immunization with the recombinant rSBm7462® and synthetic SBm7462® peptides.

Method

Twelve calves kept in arthropod-proof isolation were divided in three groups: five immunized with recombinant peptide produced in *Pichia pastoris* (rSBm7462®), five immunized with synthetic peptide SBm7462®; in inoculations was used 2 mg of peptides more 1,5 mg of saponin as adjuvant, two control immunized with 2 mg of *P. pastoris* crude extract. All groups were injected at 30 days of intervals by subcutaneous way. The challenged were with 4500 larvae 21 days after the last inoculation. Ten adult females per group were randomly selected for analyses. Using H&E was observed that recombinant and synthetic peptides caused similar histological changes in intestinal epithelial as nuclei detachment, cytoplasm vacuolation, rupture of basement membrane and cellular erosion, this pathology wasn't observed in ticks collected from controls.

Conclusions

We can infer that antibodies generated by immunization with peptides, induce changes in midgut when interact with epitopes of Bm86, affecting the endocytosis process. This interactions lead to nutritional failure, affecting metabolism and reproductive capacity in females.

0194

Research Project for Integrated Control of the Southern Cattle Fever Tick in Puerto Rico

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Objectives

Puerto Rico (PR) is infested with the southern cattle fever tick (SCFT), *Rhipicephalus* (*Boophilus*) *microplus*, which is considered the most economically important external parasite of livestock worldwide. A research coalition involving the livestock industry in PR, the PR Department of Agriculture (PR-DA), and the United States Department of Agriculture (USDA) was established to develop an integrated SCFT control program.

Method

Several technologies will be combined to mitigate the direct impact of SCFT, and its indirect effects as vector of bovine babesiosis and anaplasmosis. Novel anti-SCFT vaccine technologies researched by the USDA-Agricultural Research Service Knipling-Bushland U.S. Livestock Insects Research Laboratory (USDA-ARS-KBUSLIRL) in collaboration with animal health industry partners are pivotal to this project. The use of other technologies that recently became commercially available will be combined with an anti-SCFT vaccine. This approach addresses food safety and environmental health concerns with the ecological impact, and residue levels of synthetic acaricides in cattle products like milk. The project contemplates the implementation of good acaricide management practices through the acknowledgement of parasite economic thresholds prior to treatment and the use of novel pesticide formulations containing natural products, which are labelled for use in, and around lactating cows.

Conclusions

Through this project, dairy and beef cattle producers in PR will have access to an integrated tick control program allowing them to manage in a sustainable manner the economic impact of the SCFT on their operations as a result of the concerted efforts taking place between the animal health industry, and federal and state regulatory agencies.

0222

Evaluation of the protective ability novel antigens against the cattle tick, *Rhipicephalus microplus*, in cattle

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Objectives

Rhipicephalus microplus is one of the most successful tick species worldwide and act as an effective disease vector posing major threats due to the increased incidence of acaricide resistance. Bm86, a glycoprotein located in the tick gut epithelial cells was discovered to elicit a protective immune response in cattle, but with varying efficacy. The synergistic and/or co-immuno-stimulatory benefits of vaccinating with either complex protein extracts or tick antigen combinations have also been explored extensively in order to increase vaccine efficacy. To date, conventional yeast two-hybrid system were successfully employed to identify the interacting protein partners of Bm86.

Method

Cattle trials are underway to evaluate the three newly identified binding partners for their synergistic protective ability against South African *R. microplus* ticks, to improve the Bm86-vaccine. Tick survival and fecundity, antibody titres (via ELISA) and cattle immune responses (via DNA microarray, flow cytometry and histology) are assessed to evaluate these antigens in crossbred South African cattle strains. By isolating IgG during the trial subsequent capillary tick feeding is performed to assess and establish an *in vitro* feeding system to allow the reduction of animal studies for future antigen evaluation. Furthermore, such an approach allows studying gene expression profiles in both the host and parasite, which may elucidate the exact mechanisms of Bm86 and its interacting partners which are currently unknown.

Conclusions

The development of a vaccine would result in reduced selection pressure on ticks thus slowing the development of resistance to acaricides in addition to less environmental contamination and economic losses caused by *R. microplus*.

0230

Resistance to *Rhipicephalus* ticks in Nguni cattle reared in the semiarid areas of South Africa

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Objectives

Nguni cattle have been reported to be resistant to ticks and TBD, however, the mechanisms responsible for the trait are not fully understood. The broad objective of this study was to determine the mechanisms of resistance to ticks in Nguni cattle reared in the semiarid areas of South Africa.

Method

Relationships between tick count and skin thickness, hair length, coat score, cutaneous hypersensitivity response to unfed larval extracts (ULE) of the ticks *Rhipicephalus decoloratus* and *Rhipicephalus microplus*, and inflammatory infiltrates were assessed in seven to nine month old Nguni (n = 12) and Bonsmara (n = 12) heifers to determine the mechanisms of resistance to the ticks. Nguni heifers had lower ($P < 0.05$) coat scores, hair length and tick counts than the Bonsmara heifers. The relationship between tick counts and coat score was positive ($P < 0.05$) and linear in the Nguni ($y = 1.90x - 0.40$; $R^2 = 0.36$) and quadratic in Bonsmara ($y = -7.98x^2 + 12.74x - 3.12$; $R^2 = 0.46$) heifers. Nguni heifers presented a less ($P < 0.05$) severe immediate hypersensitivity response than the Bonsmara heifers and had a delayed hypersensitivity reaction which was absent in the Bonsmara heifers at 72 h post inoculation with ULE of *Rhipicephalus* ticks. Parasitized sites in Nguni heifers had higher ($P< 0.05$) counts of basophils, mast and mononuclear cells than those in the Bonsmara heifers. Conversely, parasitized sites in Bonsmara heifers had higher ($P< 0.05$) neutrophil and eosinophil counts than those in the Nguni heifers.

Conclusions

It was concluded that smooth and short coats, delayed type hypersensitivity and cutaneous basophil and mast cell infiltrations could be responsible for increased tick resistance in the indigenous Nguni cattle breed of South Africa.

0233

An RNAi-based approach for screening salivary protective antigens in argasid ticks.

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Objectives

RNAi gene silencing by dsRNA injection in adult ticks has proved to be a feasible method for identifying protective antigens. In ixodids, which synthesize part of its saliva components during feeding, a single injection of specific dsRNAs before tick attachment can decrease feeding performance. This doesn´t work with argasids because they have all their saliva components pre-synthesized. In argasids, an RNAi-based screening for salivary protective antigens will require consumption of the stored saliva molecules (first feeding) and, simultaneously, sustained knockdown of target genes during blood digestion until a second feeding, in which potential feeding impairing will be observed.

Method

To test this hypothesis, we knocked down several salivary antiheamostatics coding genes in first nymphal instars of *O. moubata* by three consecutive electroporations with dsRNAs of the target genes (4 days before feeding, and 24 hours and 12 days after feeding) covering thus the whole cycle of blood digestion and moulting. The newly emerged nymphs were fed and significant reductions in the amount of ingested blood were observed for up to six of the nine genes tested.

Conclusions

Electroporation can be used to deliver dsRNA into *O. moubata* nymphs and to induce RNAi-mediated gene knockdown. Three dsRNA doses are required to maintain the knockdown effect throughout the whole feeding/moulting cycle, allowing the generation of perceptible phenotypes in the next feeding event consistent with a loss of function of the targeted genes. This procedure could be applied in argasid ticks for selecting salivary antigens as vaccine targets.

0236

The potential of recombinant ferritin 1 and ferritin 2 as anti-tick vaccine against *Haemaphysalis longicornis*

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Objectives

We have previously reported that the hard tick *Haemaphysalis longicornis* has two kinds of the iron-binding protein ferritin, an intracellular ferritin 1 (HlFER1) and a secretory ferritin 2 (HlFER2), which are both physiologically important in blood feeding and reproduction, and protection against oxidative stress. Here, we investigated the potential of targeting ferritin for tick control by immunizing the host with recombinant ferritins.

Method

Rabbits were immunized with 100 µg recombinant HlFER1 or HlFER2 mixed with equal volume of adjuvant for three times subcutaneously, two weeks apart each injection. Control rabbit was immunized with adjuvant alone. Antisera were collected at 0, 1, 3, and 5 weeks to check antigen-specific antibody titer by serum ELISA. Two weeks after the final immunization, 30 adult female ticks were infested on the ears of the rabbits and allowed to feed to repletion. After dropping, ticks were weighed, and then monitored for survival, egg laying and subsequent hatching. The antibody titer of HlFER1-immunized rabbit greatly increased after second administration, while that of HlFER2-immunized rabbit gradually increased until third administration. Cross-immunity was also observed. Significantly lower bodyweight was observed in the ticks infested from HlFER2-immunized rabbit compared to those from control rabbit. Immunization using recombinant HlFER2 resulted to more significant reduction in ticks' egg laying and subsequent hatch.

Conclusions

Vaccination using recombinant HlFER2 showed higher efficacy than using recombinant HlFER1, with marked effects on egg laying and hatch. These results suggest that HlFER2 may be a potential antigen for tick control.

0238

Assessment of the protective efficiency of the *Ornithodoros moubata* apyrase and savignyigrin orthologues as vaccine targets

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Objectives

Tick salivary apyrase and the *Ornithodoros savignyi* desintegrin savignyigrin are antihaemostatic agents that prevent platelet aggregation at the tick bite site thus allowing the parasite to complete its blood feeding. Recently, we have found significant amounts of apyrase in the *Ornithodoros moubata* saliva, as well as apyrase and savignyigrin orthologs in a cDNA expression library from the salivary glands of *O. moubata*. We have also observed that the RNAi-mediated knockdown of the apyrase and savignyigrin-like genes decreases feeding performance by more than 30%, strongly suggesting that both proteins could be interesting candidates to be targeted by anti-tick vaccines.

Method

To assess the protective efficacy of the *O. moubata* apyrase and savignyigrin orthologs as vaccine antigens we have cloned and produced them as recombinant proteins, and administering them to rabbits in Freund's adjuvants. Ticks specimens fed on the vaccinated rabbits experienced 30% - 40% reductions in feeding performance, and 26% - 39% reductions in female fertility. Vaccine-induced mortality rates were insignificant.

Conclusions

The recombinant forms of salivary apyrase and savignyigrin orthologs of *O. moubata* induce protective immune responses in rabbits reaching up to 41,4 % efficacy, which make them promising candidates to be added to the current repertory of anti-tick vaccine targets.

0239

Ornithodoros moubata salivary secreted phospholipase A2 (PLA2): a new P-selectin antagonist ligand and vaccine target.

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Objectives

The *O. moubata* Om44 protective antigen is an unidentified salivary molecule that binds host P-selectin and blocks its downstream haemostatic activities. To identify Om44, NAPPA microarrays were produced from an *O. moubata* salivary gland cDNA expression library and screened for P-selectin ligands. A secreted PLA2 was recognized by both the recombinant P-selectin/IgG chimera and the anti-Om44 hyperimmune rabbit serum used as probes, suggesting that PLA2 was the Om44 identity. The objective of this work was to confirm the results of the microarray analyses and assess the protective efficacy of recombinant PLA2 against *O. moubata*.

Method

The cDNA coding sequence of PLA2 was cloned into two expressing vectors and produced in two recombinant versions: *in vivo* in *E. coli* cells, and *in vitro* using a IVTT cell free system that allows post-translational modifications, the 1-Step Human Coupled IVT-Kit. Both recombinant forms were recognized by the anti-Om44 serum and by the recombinant P-selectin/IgG chimera by ELISA and western blot. In addition, the recombinant PLA2 obtained in bacterial cells was administered to rabbits with Freund's inducing protective responses that reduced tick feeding and fertility (44,5% protective efficacy).

Conclusions

The *O. moubata* salivary secreted PLA2 is a P-selectin antagonist ligand with anti-haemostatic activity during tick feeding. PLA2 blockage with vaccine-induced antibodies impairs tick feeding and reproduction making this salivary molecule a promising vaccine target.

0253

Immunogenic potential of the recombinant *Rhipicephalus microplus* aquaporin protein against the tick *Rhipicephalus sanguineus* Latreille, 1806 in domestic dogs.

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Objectives

Aquaporins allow water transport regulation through the highly hydrophobic lipid bilayer of cell membranes. As ticks ingest large volumes of host blood in relation to their size, they are required to concentrate blood components and have efficient water transport mechanisms. This study aimed to evaluate in domestic dogs the immunogenic potential of a recombinant Rhipicephalus microplus aquaporin protein (Antigen 1) followed by challenge infestation with *R. sanguineus*.

Method

Dogs were distributed into two experimental groups of five animals each: G1- immunized thrice by intramuscular route with 1 mL of 100 mg of Antigen 1 plus Montanide at three weeks interval; G2- treated with an equal volume of Montanide. Tick challenge infestation was performed 21 days after the third immunization. Antibody titers against Antigen 1 of dog´s sera through ELISA and biological parameters of all stages of *R. sanguineus* were used for comparisons between G1 and G2. Immunized dogs developed effective antibodies against Antigen 1. After challenge infestation, although larvae from G1 presented 8.7% longer engorgement period than larvae from G2, they weighed 7.2% less. Moreover, nymphs from G1 demonstrated significant differences when compared to nymphs from G2 as they weighed 3.6% less and presented 4.5% lower engorgement period. Finally, though the mean engorged female weight in both groups was not different statistically, females from G1 presented 12% reduction in their engorgement periods when compared to the ones from G2.

Conclusions

Results point out that Antigen 1 may be a potential immunogen against *R. sanguineus* infestations in domestic dogs.

0270

The impact of a imidacloprid/ flumethrin collar on the transmission of *Babesia canis* by *Dermacentor reticulatus* ticks to dogs.

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Objectives

The vector capacity of ticks in the transmission of a variety of pathogens to dogs is challenging in all day veterinary practice. Preventative care to avoid tick- host interaction and subsequently avoidance of pathogen transmission during tick feeding is today's best-practice. As the tick species and thus tick-borne diseases different worldwide, today's international travel of pet owners with their companion animal and translocation of dogs from infected thus endemic regions to non-endemic regions (re-homing practice) is causing new emerging and re-emerging disease threats.

Method

A total of 16 (9 male and 7 female) dogs were allocated to two groups of 8 dogs each. One group was treated with imidacloprid/flumethrin collar 28 days prior to *Dermacentor reticulatus* infestation, infected with *Babesia canis*. A second group served as negative controls. Tick counts were performed daily until day 6 post infestation. Blood samples were collected for PCR and serology (IFA) testing on days 14, 21 and 28 post infestation. For animal welfare dogs developing clinical signs of babesiosis were rescued and treated appropriately. Tick efficacy was calculated for each group and assessment day according to international guidelines. The ability of the collar to prevent transmission of *B. canis* in the treated group was compared to an untreated control group. All 8 dogs in the untreated control group became infected, which were detected in blood smears as early as day 6 post tick-application. All control dogs developed clinical signs of babesiosis and were rescue and treated. These dogs became positive in serology and confirmed PCR/RLB positive. None of the 8 dogs treated with the imidacloprid/flumethrin collar became infected with *B. canis*, which was confirmed by the absence of specific *B. canis* antibodies and DNA as confirmed by PCR/RLB.

Conclusions

The imidacloprid/ flumethrin collar caused 96.02% of the ticks to die within 48 hours post infestation and this increased to 100% within 4 days. Despite the high percentage of 44% of the Dermacentor ticks were infected with *B. canis*, they were unable to transmit the pathogen to the treated group. Therefore the imidacloprid/flumethrin collar effectively prevented transmission of *B. canis* within the initial first month after collar application onto the dogs.

0049

Salivary gland transcriptome of *Rhipicephalus (Boophilus) microplus*

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Objectives

The cattle tick, *Rhipicephalus (Boophilus) microplus* is a tick of veterinary importance globally transmitting *Babesia bovis* and *B. bigemina.* Control of ticks is important and needed to prevent livestock diseases caused by tick-transmitted pathogens. Tick control measures have relied on the use of acaricides; however this has several disadvantages including among others the development of acaricide-resistant ticks, the cause of environmental pollution and milk and meat contamination. Therefore, there is a need for alternative method and vaccines directed against tick feeding are considered as best option in an integrated pest control.

Method

Female ticks were collected at five different feeding stages. Purified salivary gland RNA was used to synthesize five cDNA libraries which were then sequenced with Illumina MiSeq technology. Data was assembled using CLC Genomics Workbench, Trinity and Minia. BLASTX analysis of open reading frames indicated the presence of major secretory protein families such as Kunitz, lipocalins, serpins and metalloproteases, while the majority of transcripts coded for housekeeping genes. Analysis of the datasets indicates differential gene expression.

Conclusions

Transcripts will be selected based on expression profiles, abundance and bioinformatics analysis and will be validated by proteomic approaches and functionally characterized. Promising protein candidates will be tested in vaccination trials.

0271

Human Pathogens Associated with ticks in Australia

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Objectives

Australian ticks transmit several pathogens related to human infections. Firstly, Q Fever, caused by *Coxiella burnetii*, is the most common. Ticks known to carry this bacterium include: *Amblyomma triguttatum* (ornate kangaroo tick); *Haemaphysalis humerosa* (bandicoot tick); *Ixodes holocyclus* (paralysis tick); *Bothriocroton auruginans* (wombat tick) and possibly others. However, most human cases of Q Fever are transmitted by aerosol spread from infected cattle, sheep and goats. Secondly, Rickettsial diseases are the most common tick-transmitted human infections. *I. holocyclus* and *I. tasmani* (common marsupial tick) transmit *Rickettsia australis*, causing "Queensland Tick Typhus". *B. hydrosauri* (southern reptile tick) transmits *R. honei*, causing "Flinder's Island Spotted Fever". *H. novaeguineae* transmits *R. honei*, subspecies *marmionii*, causing "Australian Spotted Fever". *A. triguttatum* contains *R. gravesii* and may transmit it to humans causing infection. Thirdly, rare infection/allergic reaction to *Ornithodoros capensis* (bird tick) containing the flavivirus "Saumarez Reef Virus" has also been reported. Lastly, an unknown tick vector transmitting *Babesia microti* causing human babesiosis (one case only) has been reported.

Method

To date, there have been no human infections from ticks colonized with Ehrlichia spp, *Anaplasma* spp or *Borrelia* spp (including un-confirmed cases of Lyme Disease). Such ticks may not occur in Australia.

Conclusions

These infections, their epidemiology and associated tick vectors, will be discussed.

NOTES: